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(54) Title: NEOPEPTIDES AND METHODS USEFUL FOR DETECTION AND TREATMENT OF CANCER

(57) Abstract: The present invention relates to neopeptides and methods useful for the detection and treatment of disorders associ-  
ated with frameshift mutations in coding microsatellite regions. The neopeptides and methods are applicable in cancers, especially  
of DNA mismatch repair deficient (MMR) sporadic tumours and HNPCC associated tumours. The neopeptides disclosed in the in-  
vention are useful for detection of disorders and in therapy such as e.g. immuno-therapy. The diagnostic methods relate to diagnosis  
and prognostic assessment of disorders associated with frameshift polypeptides originating from frameshift mutations in coding mi-  
crosatellite regions of genes based on the detection of immunological entities directed against said frameshift polypeptides in body  
fluids. With respect to the treatment of cancer, especially of DNA mismatch repair deficient (MMR) sporadic tumours and HNPCC  
associated tumours, the invention pertains to methods which use immuno therapy with combinatorial mixtures of tumour specific  
frameshift peptides to elicit a cytotoxic T-cell response specifically directed against tumour cells for use in prevention as well as in  
curative treatment of cancers and precancers.

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**Compounds and methods useful for detection and treatment of cancer**

The present invention relates to compounds and methods useful for the detection and treatment of disorders associated with frameshift mutations in coding microsatellite regions. The compounds and methods are applicable in cancers, especially of DNA mismatch repair deficient (MMR) sporadic tumours and HNPCC associated tumours. The compounds disclosed in the invention are useful for detection of disorders and in therapy such as e.g. immuno-therapy. The diagnostic methods relate to diagnosis and prognostic assessment of disorders associated with frameshift polypeptides originating from frameshift mutations in coding microsatellite regions of genes based on the detection of immunological entities directed against said frameshift polypeptides in body fluids. With respect to the treatment of cancer, especially of DNA mismatch repair deficient (MMR) sporadic tumours and HNPCC associated tumours, the invention pertains to methods which use immuno therapy with combinatorial mixtures of tumour specific frameshift peptides to elicit a cytotoxic T-cell response specifically directed against tumour cells for use in prevention as well as in curative treatment of cancers and precancers.

Tumour cells accumulate mutations in components of cellular pathways, that are essential for the maintenance of normal growth and differentiation. In human epithelial tumours, 2 types of genetic instability have been identified: chromosomal instability (CIN), which marks structural and numerical chromosomal aberration in aneuploid neoplastic cells, and microsatellite instability (MSI), which reflects length variations at repetitive DNA sequences in diploid tumour cells. The type and spectrum of mutated genes markedly differs among CIN and MSI tumours, suggesting distinct but not mutually exclusive pathways of carcinogenesis. MSI occurs in about 90% of hereditary nonpolyposis colorectal cancers (HNPCC) as well as in about 15% of sporadic tumours of the colon and other organs, and is caused by mutational inactivation of different DNA mismatch repair genes.

The mutations lead especially in the case of frameshift mutations in coding microsatellite regions to the expression of new peptide sequences in the affected cells, that do not occur in wild-type cells. The altered peptides may be used as detection markers for disorders associated with frameshift mutations in coding microsatellite regions such as degenerative disorders or cancers (e.g. gastrointestinal cancers).

The accumulation of genetic alterations and resulting mutant proteins represent a major obstacle for tumour cells to escape immune surveillance. The mutant proteins or peptides encoded by expressed mutant genes may elicit a specific cellular immune response and thus may be recognized by CTL. This is especially true concerning mutations resulting from chromosomal instabilities, but also pertains to more subtle genetic alterations, such as small deletions and insertions in microsatellites.

This situation can be used for prevention and therapies in cancers. The cancer cells are characterized by the expression of neo-peptides, which arise from the genetic alterations. These proteins are not present in normal, non cancerous tissues. Thus, the neo-peptides may be used to distinguish on a molecular level between tumours and normal tissues. As tools suitable for the molecular discrimination antigen specific molecules or cells may be used. Thus it is possible to elicit an immune response against frameshift peptides, as might arise from MSI mutations, specific for tumours. Using cytotoxic T lymphocytes or FS8-specific antibodies it is thus possible to specifically attack and eliminate tumour cells in organisms and tissues.

A large number of genes containing coding microsatellites have been identified. Various of these genes show mutations within the microsatellites with certain frequencies in sporadic tumours. For few of these genes it could be shown, that they are involved in the majority of cases of particular tumours. For example the (A)<sub>10</sub> tract within the TGF $\beta$ RII gene and the (G)<sub>8</sub> tract within the BAX gene are commonly mutated in gastrointestinal cancers such as colon cancer or gastric cancer.

The T-cell mediated immune response on the other hand provides a strong and specific selection pressure for the rapidly growing tumour cells. (Tomlinson I, et al., Nat. Med. 1999; 5: 11-2) Thus tumour evolution is forced to circumvent the immune surveillance by the cellular immune response. As a consequence of this selection pressure, mutations in genes of the antigen processing or presenting machinery arise in MMR-deficient tumour cells. In fact evasion of the immune surveillance by acquiring  $\beta$ 2-microglobulin mutations has been observed at high frequency in MSI<sup>+</sup> tumour cells (Bicknell DC, et al., Curr. Biol 1996; 6: 1695-7). Other targets of specific mutation or down-regulation of expression are TAP1/TAP2 or HLA alleles. Direct down-regulation of expression of immunogenic epitopes is also a possible mechanism of immune escape as has been shown for melanoma-associated antigens in vivo (Jager E, et al.,

Int. J. Cancer 1997; 71: 142-7). Due to this fact<sup>3</sup> the relevant epitopes may not be detectable by the immune system.

- As a consequence the promising therapies based on immune response directed against frameshift peptides in tumour tissues may fail in a not yet determined number of cases of tumours. So the approaches for the use of the vaccination therapy of cancer mediated by frameshift peptides might be limited by the fact, that the potential immunogenic frameshift peptides are not always detectable by the immune system due to mutations in the antigen presenting and processing properties of the cancer cells.
- 10 Even supposed, that during specific stages of tumour development cells express the aberrant proteins and thus are vulnerable to the immune surveillance, there potentially remains a population of tumour cells, that is not eliminated by the therapy. This state is quite undesirable for any therapy of cancers, for the remaining cells may continue growing and thus the tumour is not eliminated from the organism.
- 15 Similarly methods for detection of molecular markers suitable for diagnosis of disorders associated with frameshift mutations in coding microsatellite regions and for assessment of prognosis for said disorders are prone to overlook especially markers originating from small populations of affected cells as they may occur particularly in early stages of the disorder. This may in part be overcome by an elevated effort in the
- 20 preparation of testing samples to raise the probability of the detection of disorders. However especially concerning disorders located in body regions, that are merely inaccessible, or accessible only under circumstances, that are quite consuming or uncomfortable to patients alternative methods for reliable detection of molecular markers associated with disorders are desirable.
- 25 It is known in the art, that the mutant proteins or peptides derived from aberrantly expressed proteins may elicit a specific cellular immune response and thus may be recognized by cytotoxic T lymphocytes (CTL). This is especially true concerning mutations resulting from chromosomal instabilities, but pertains also to the more subtle genetic alterations, such as small deletions and insertions in microsatellites.
- 30 The T-cell mediated immune response on the other hand provides a strong and specific selection pressure for the rapidly growing tumour cells (Tomlinson I, et al., Nat. Med. 1999; 5: 11-2). Thus tumour evolution is forced to circumvent the immune surveillance by the cellular immune response. As a consequence of this selection

pressure, mutations in genes of the antigen processing or presenting machinery arise in DNA mismatch repair (MMR)-deficient tumour cells. In fact evasion of the immune surveillance by acquiring  $\beta$ 2-microglobulin mutations has been observed at high frequency in MSI<sup>+</sup> tumour cells (Bicknell DC, et al., Curr. Biol 1996; 6: 1695-7). Other targets of specific mutation or down-regulation of expression are TAP1/TAP2 or HLA alleles. Direct down-regulation of expression of immunogenic epitopes is also a possible mechanism of immune escape as has been shown for melanoma-associated antigens in vivo (Jager E, et al., Int. J. Cancer 1997; 71: 142-7). Due to this fact the relevant epitopes may not be detectable by the immune system. Thus it was suspected, that components of the immune system directed against novel peptides, characteristic for e.g. tumour cells, are not suitable for the detection of the disorders especially in the case of frameshift mutation peptides. This is due to the fact, that MSI disorders are frequently associated with DNA mismatch repair deficiency and thus are especially prone to mutations. This makes the affected cells especially apt to escape mutations in response to the attack by the immune system.

The inventors have now surprisingly found, that specific antibodies or antigen recognizing cells directed against a particular new frameshift peptide are in detectable levels present within the body fluids of individuals harbouring MSI associated disorders. This is especially due to the fact, that during specific stages of tumour development cells express the aberrant proteins. During these stages the peptides may be accessible to the immune surveillance, and thus an immune response may be elicited. Even though cell populations affected by respective disorders such as tumours may be eliminated from the organism, or may be mutated, such, that no further presentation of immunogenic epitopes may occur, there remains an immunological memory of the presence of the respective peptides at a certain time. Such the evolution of a cell population affected by a disorder associated with the expression of new frameshift peptides leaves immunological traces of its existence consisting e.g. of antibodies and specific T-lymphocytes directed against the particular peptides. Hence the presence of specific immune response elements directed against a particular frameshift peptide is indicative of the presence of a population of (tumour) cells, that expresses or has expressed at a certain time the respective peptide.

To further enhance the fidelity of the detection of the presence or absence of a disorder a set of peptides frequently occurring in disorders such as tumours may be applied in the detection reaction.

The present invention thus provides a method for the detection of disorders associated with frameshift peptides arising from mutations in coding microsatellite regions based on the detection of specific immunological entities directed against said frameshift peptides present in the body fluids of affected individuals directed against said frameshift peptides. The method is suited for primary detection of disorders such as tumours, for the early detection of disorders or of precursory stages of disorders, and for the assessment of prognosis in said disorders.

One further aspect of the invention is based on the inventors findings, that a mixture of frameshift peptides chosen according to combinatorial parameters minimizes the escape of particular populations of tumour cells from immunogenic elimination and may be used as a vaccine against a wide variety of DNA mismatch repair deficient tumours. The invention is based on the fact, that the immune escape of tumour cells is directed towards single immunogenic epitopes (frameshift peptides) in a potentially restricted subpopulation of the tumour cells. In the vast majority of MSI+ tumours various mutations may be found within the genome of the affected cells. So a combinatorial vaccination approach including several antigenic peptides could overcome this obstacle.

It is one aspect of the present invention to provide nucleic acid sequences of the genes TAF1B, MACS, UVRAG, ELAVL3, TCF6L1, ABCF1, AIM2, CHD2 and HT001, that have frameshift mutations within their coding regions.

A second aspect of the present invention is to provide new frameshift peptides, that occur in a wide range of different MSI+ tumours.

A third aspect of the present invention is a method for detection of MSI+ tumours using said frameshift peptides as molecular markers.

A fourth aspect of the present invention is a method for treatment of MSI+ tumours using said frameshift peptides for therapeutic purposes.

In a fifth aspect the present invention provides sets of frameshift peptides, that occur in a wide range of different MSI+ tumours and that diminish the probability of escape of tumours to be attacked by the immuno-therapy.

A sixth aspect of the present invention is a method for vaccination against MSI+ tumours using said set of frameshift peptides.

A seventh aspect of the present invention is a method for treatment of MSI<sup>+</sup> tumours using a set of frameshift peptides, that elicit an immune response directed against a wide range of tumours.

An eighth aspect of the invention relates to the detection of disorders associated with frameshift mutations in coding microsatellite regions comprising the detection of immunological entities directed against frameshift peptides in body fluids of individuals.

The present invention thus provides compounds and methods for the therapy and detection of disorders associated with frameshift peptides arising from mutations in coding microsatellite regions. The diagnostic and therapeutic methods are suited for application in disorders such as tumours or in precursory stages of disorders.

Within the context of the present invention disorders associated with frameshift mutations comprises for example degenerative diseases, such as neurodegenerative diseases, vascular disorders, disorders caused by stress, such as oxidative stress, chemically induced stress, irradiation induced stress, etc. and cancers including all sporadic cancers as well as HNPCC associated cancers. Cancers as used herein may comprise e.g. colorectal cancer, small cell lung cancer, liver cancer (primary and secondary), renal cancer, melanoma, cancer of the brain, head and neck cancer, gastrointestinal cancers, leukemias, lymphomas. prostate cancer, breast cancer, ovary cancer, endometrial cancer, lung cancer, bladder cancer etc..

The method according to the present invention may be applied to any eucaryotic organisms. In one aspect the eucaryotic organisms are those exhibiting an immunologic defense system. The eucaryotic organisms are for example mammalian animals and especially animals of agricultural value such as pigs, cows, sheep, etc., companion animals, such as cats, dogs, horses etc., animals employed in research purposes such as mice, rats, rabbits, hamsters etc. or humans.

Nucleic acid molecules according to the present invention may comprise polynucleotides or fragments thereof. Preferred polynucleotides may comprise at least 20 consecutive nucleotides, preferably at least 30 consecutive nucleotides and more preferably at least 45 consecutive nucleotides of the sequences. The nucleic acids according to the present invention may also be complementary or reverse complementary to any of said polynucleotides. Polynucleotides may for example include single-stranded (sense or antisense) or double-stranded molecules, and may be DNA (genomic, cDNA or synthetic) or RNA. RNA molecules comprise as well

hnRNA (containing introns) as mRNA (not containing introns). According to the present invention the polynucleotides may also be linked to any other molecules, such as support materials or detection marker molecules, and may, but need not, contain additional coding or non-coding sequences.

- 5 Mutation as used in the context of the present invention may comprise insertions or deletions of one or several nucleotides (or nucleotide repeats) within the specified microsatellite sequences. In a preferred embodiment of the present invention the number of nucleotides to be inserted or deleted is not 3 or must not be divisible by 3, such that the mutation leads to a frameshift with respect to the translational reading
- 10 frame of the downstream nucleic acid sequence. Thus the nucleic acid sequence downstream of the mutation point will render a polypeptide-sequence different from the native sequence encoded by the respective gene. The mutation in these cases leads to a novel peptide sequence (a neo-peptide). Commonly the new peptide sequence is short due to the fact, that novel stop codons arise from the shift in the reading frame.
- 15 Frameshift mutations in microsatellites are usually due to DNA polymerase slippage and may be characterized by the type of the repeat. Thus in mono nucleotides repeats this type of mutation renders 1nt insertion or deletion. In dinucleotide repeats and tetranucleotide repeats mutations are insertions or deletions of 2 or 4 nt respectively. For example commonly (-1) mutations occur in mononucleotide repeats (MNRs). In
- 20 these mutations one nucleotide is deleted such that the reading frame is shifted by one nucleotide toward the 5' end of the gene compared to the original reading frame. This type of mutation renders a reading frame identical to that produced by (+2) mutations, which arise from two nucleotide insertions in the respective microsatellites. The respective (-1) or (+2) polypeptides might differ by one amino acid. The other
- 25 frameshift mutation variant leading to frameshift mutations differing from (-1) mutations concerning the resulting reading frame is the (+1) mutation, arising from one nucleotide insertion, thus rendering a reading frame one nucleotide toward the 3' end of the gene compared to the original reading frame. This mutation type gives a reading frame identical to that of (-2) mutations, wherein the encoded polypeptides differ by one
- 30 amino acid. (-3) and (+3) mutations are irrelevant according to the present invention, for they do not give rise to frameshift polypeptides.

Frameshift polypeptides as used herein shall comprise any polypeptides or fragments thereof, that arise by a frameshift mutation within a coding microsatellite sequence of a



gene. A gene may harbour one or more coding microsatellite regions, that may give rise to frameshift peptides. The coding microsatellites according to the present invention comprise mononucleotide repeats, dinucleotide repeats, trinucleotide repeats, tetranucleotide repeats and pentanucleotide repeats of any length. According to present invention coding microsatellites contain preferably at least 3 and more preferably at least 5 repeats of the respective nucleotide pattern (1-5 nucleotides, which are repeated).

The frameshift polypeptides as used according to the present invention comprise at least 1, more preferred at least 2 and even more preferred at least 3 amino acids of the mutated part of the polypeptide. Additionally, the frameshift polypeptides may comprise fragments of the originally non-mutated proteins and/or may be fused to any other polypeptide sequences suitable for the purposes of the present invention. Examples of such polypeptide sequences are linker sequences, or structural peptide sequences, such as beta barrels, loop sequences etc. that facilitate the immunogenicity of the frameshift sequences according to the present invention within the fusion polypeptide.

In certain embodiments of the invention the frameshift polypeptides suitable for the methods disclosed herein are immunogenic polypeptides. This requires, that the polypeptides may stimulate immune responses in host organisms either in the form the polypeptides adopt in their natural environment and/or especially in the form the polypeptides adopt after processing by the cellular antigen processing and presenting machinery.

According to the present invention the frameshift polypeptides may also be represented by nucleic acids coding for said polypeptides. These nucleic acids may for example be used for the *in situ* expression of the respective polypeptides. For the purpose of expression of nucleic acids, the particular nucleic acids may be joined with suitable other nucleic acid sequences, that enable for the cloning and expression of said nucleic acids encoding the frameshift polypeptides.

In certain embodiments of the present invention frameshift polypeptides may comprise fusion or chimeric polypeptides containing sequences disclosed herein. Fusion proteins comprise the frameshift polypeptide according to the present invention together with any second and further polypeptides, such as e.g. one more frameshift polypeptide of the same sequence or of another sequence. Heterologous polypeptides may comprise enzymes, receptor molecules, antigens, antigenic or immunogenic

epitopes or fragments, antibodies or fragments thereof, signalling polypeptides or signal transducing polypeptides, labelled polypeptides etc.. In one embodiment of the invention the fusion peptides may be constructed for enhanced detection or purification of the frameshift polypeptides, or of complexes of the frameshift polypeptides with the respective immunological entities according to the present invention. For the purpose of purification tags, such as e.g. his-tags, myc-tags etc. may be added to the polypeptides. For the purpose of detection antigenic portions, enzymes, chromogenic sequences etc. may be fused to the polypeptides. The fusion proteins of the present invention may (but need not) include a linker peptide between the first and second polypeptides.

A nucleic acid sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate nucleic acid sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a nucleic acid sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a nucleic acid sequence encoding the second polypeptide ensuring the appropriate reading frames of the sequences to permit mRNA translation of the two nucleic acid sequences into a single fusion protein that retains the biological activity (antigenicity) of both the first and the second polypeptides.

A peptide linker sequence may be employed to separate the first and the second polypeptides by a distance sufficient to ensure, that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., Gene 40:39-46, 1985; Murphy et al., Proc. Natl. Acad. Sci. USA 83:8258-8262, 1986; U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180. The linker sequence may be from 1 to about 50 amino acids in length. Peptide sequences are not required when the first and second

polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

In certain embodiments of the present invention, especially for the detection of specific antibodies directed against frameshift polypeptides, the frameshift polypeptides themselves may be employed. Immunogenic portions as used herein is a portion of a protein, that is recognized by a B-cell and/or T-cell surface antigen receptor. The immunogenic portions comprise at least 5 amino acid residues, more preferably at least 10 amino acid residues and most preferably at least 15 amino acid residues of the frameshift polypeptides according to the present invention.

Immunogenic portions useful for the detection of specific antibodies may be provided as oligopeptides or as part of larger proteins. This is dependent on the embodiment of the invention. Where antibodies are to be detected, the antigenic epitopes may either be primary structures of polypeptides or the epitopes may be built by complex arrangements of tertiary structures of polypeptides. Concerning cells directed against specific frameshift peptides the relevant immunogenic portions are merely short fragments of peptides with a length of about 10 – 20 amino acids. Thus depending on the particular detection method the immunogenic portions have to be chosen.

In one embodiment of the invention a set of frameshift polypeptides is used for the detection of antibodies. The set may be a combination of the relevant peptides in solution, in cases, where information about the presence of immunological entities in general is sought. In contrast, when information about the presence or absence of particular frameshift peptides within a set of peptides is sought, the frameshift peptides may for example be tested each as a single, simultaneously in multiple testing reactions. Such experiments may for example be carried out in form of multi-well tests or using peptide arrays etc.

In order to address a representative choice of mutations potentially characterizing a disorder a set of frameshift polypeptides used according to the method disclosed herein comprises for example 5-20, in a preferred embodiment 10-30, in another preferred embodiment 20-50, in a more preferred embodiment 50-100, in an even more preferred embodiment 100-500 and in the most preferred embodiment more than 500 different frameshift polypeptides originating from frameshift mutations in coding microsatellite regions. The frameshift polypeptides to be used as a set according to the present invention are selected with respect to a number of parameters characterizing

said polypeptides. A nucleic acid sequence encoding a fusion protein of the present invention is constructed using known recombinant DNA techniques to assemble separate nucleic acid sequences encoding the first and second polypeptides into an appropriate expression vector. The 3' end of a nucleic acid sequence encoding the first polypeptide is ligated, with or without a peptide linker, to the 5' end of a nucleic acid sequence encoding the second polypeptide ensuring the appropriate reading frames of the sequences to permit mRNA translation of the two nucleic acid sequences into a single fusion protein that retains the biological activity (antigenicity) of both the first and the second polypeptides.

- Generally using an immense or unlimited number of occurring frameshift peptides it would be possible to address any potential number of disorder, that is associated with frameshift mutations. Due to practical and immunological concerns the number of peptides, that are included in a vaccine must be limited. To ensure a broad range of impact for any diagnostic or therapeutic method employing a set of frameshift polypeptides, the selection of particular peptides has to be based on rationale considerations.

A set of frameshift polypeptides used according to the method disclosed herein comprises at least 3, 4, 5, 6 or even 7 and in certain embodiments 8, 9 or 10 frameshift polypeptides. Due to immunological as well as practical concerns The set of frameshift peptides used as a vaccine may not include an unlimited number of frameshift peptides. Preferably the set of frameshift peptides comprises at maximum 15, in a more preferred embodiment at maximum 20 and in the most preferred embodiment of the invention at maximum 30 frameshift polypeptides. In order to ensure a maximum range of disorders to be addressed by the selected set of frameshift polypeptides the members of the set have to be selected by reasonable considerations. The frameshift polypeptides to be used as a set according to the present invention are selected with respect to a number of parameters characterizing said polypeptides.

In one embodiment the members of the set are chosen, so that only three different peptides may be used to cover a wide range of tumours for as well therapeutic as well as diagnostic approaches. Using larger panels of frameshift peptides the stringency to the choice of individual peptides is lowered. In especially small sets all parameters influencing the efficacy of addressing a wide range of tumours need to be optimally

met. In larger panels even peptides occurring with lower frequencies or allowing for efficient immune-escape may be included.

One crucial aspect influencing the selection of the frameshift peptides is the mutation frequency of the relevant microsatellite region and thus the frequency of a particular expressed frameshift polypeptide. Mutation frequency as used in the context of the present invention pertains to the percentage of samples within a defined range of total samples, which show a particular mutation. Any method suitable for the determination of the percentage of individuals in a range of samples displaying the existence of a particular genotype or phenotype (with respect to the expression of polypeptides) may be employed for the determination of the frequency according to the present invention. The frequencies may be determined e.g. as described below in example 1.

The frequency may be the frequency of frameshift mutation within a coding microsatellite region or a frequency of expression of a frameshift polypeptide. Furthermore the frequency may be e.g. a frequency over a total of different tumour entities, the frequency with respect to a particular tumour entity, a frequency over several tumours entities restricted to particular stages of tumourigenesis or a frequency with respect to a particular tumour entity restricted to particular stages of tumourigenesis.

The frequency according to the present invention has to be determined taking into account a range of samples sufficient to render significant data. For example preferably at least 50 to 100 tumours may be included in the range of samples analysed. In case, that a smaller number of samples has been taken into account for determination of the frequency, a variation of the determined frequency may take place, if the range of samples is broadened.

Generally any frequency as determined may be used as a tool for the choice of a set of frameshift polypeptides according to the present invention. To ensure best results for the method according to the present invention a largest possible number of samples has to be taken into account. Yet if there are only rare data, a frequency may also be determined with respect to a restricted number of samples. This may especially be true, if the data for the restricted population of samples indicates a quite high frequency of a particular peptide, and thus implies a high therapeutic value for the respective peptide. Especially in cases, where a frequency is to be determined related to a

particular tumour entity or related to particular stages of the tumorigenesis, the population of samples may be restricted.

The mutation may occur at any time during the tumour evolution and may be persistent or may be eliminated from the genome. Thus the frequency may comprise the frequency of the expression of a peptide at a particular stage of tumorigenesis or the occurrence of a genetic mutation at a particular defined stage of tumorigenesis. In one embodiment of the invention the frequency for the mutation is determined taking into account the widest possible range of samples. In this embodiment the method disclosed herein may be especially useful for the preventive vaccination of tumours. In another embodiment of the present invention the frequencies of mutation are related to specified tumour entities. In this embodiment the method according to the present invention may e.g. especially be useful for immuno-therapeutic approaches in treatment of tumours or in the preventive vaccination of particular subpopulations with an elevated risk for the occurrence of particular tumours. In a third embodiment of the present invention the frequency may be related to particular stages in tumorigenesis of particular tumours. This embodiment may be e.g. especially useful for the treatment of diagnostically defined tumours or for adjuvant treatment of tumours simultaneously or following primary treatment of tumours.

Using frameshift polypeptides, that occur with a high frequency, the probability, that a particular tumour expresses the peptide and may thus be recognized by antibodies or antigen-recognizing cells, increases. By combination of multiple such polypeptides, the probability to address particular tumour cells further is raised. Preferred frameshift polypeptides according to the present invention occur in at least 25%, more preferred frameshift polypeptides in at least 30% and most preferred frameshift polypeptides in at least 33% of the cases of the particular condition to be treated by the method according to the present invention.

According to the present invention the polypeptides are chosen to be expressed with a high frequency, thus the set of polypeptides may be limited to a number of members and nonetheless may cover a range of occurring diseases as wide as possible. For example a set of 10 polypeptides, each occurring with a frequency of more than 30 percent will statistically cover a range of more than 95% of the potentially existing disorders, as far as they have been included in the studies leading to the respective frequencies. Using 7 polypeptides requires the application of polypeptides with higher

frequencies in order to cover a range of about 95% of potentially existing tumours. Using a set of polypeptides derived from enormously frequent mutated microsatellite regions may also allow for the employment of a set of only five different polypeptides without lowering the range of potentially addressed tumours. Thus the set of polypeptides comprises in a preferred embodiment at least 5, in a more preferred embodiment at least 7 and in the most preferred embodiment at least 10 different frameshift polypeptides.

A second aspect influencing the choice of a suitable set of frameshift polypeptides concerns the type of mutation found in the microsatellite region. Frameshift mutations microsatellites are usually due to DNA polymerase slippage and may be characterized by the type of the repeat. Thus in mono nucleotides repeats this type of mutation renders 1nt insertion or deletion. In dinucleotide repeats and tetranucleotide repeats mutations are insertions or deletions of 2 or 4 nt respectively. (Polypeptides encoded by genes with coding microsatellites and the respective polypeptides encoded by genes with frameshift mutations are given in Figure 5) For example commonly (-1) mutations occur in mononucleotide repeats (MNRs). In these mutations one nucleotide is deleted such that the reading frame is shifted by one nucleotide toward the 5' end of the gene compared to the original reading frame. This type of mutation renders a reading frame identical to that produced by (+2) mutations, which arise from two nucleotide insertions in the respective microsatellites. The respective (-1) or (+2) polypeptides might differ by one amino acid. The other frameshift mutation variant leading to frameshift mutations differing from (-1) mutations concerning the resulting reading frame is the (+1) mutation, arising from one nucleotide insertion, thus rendering a reading frame shifted one nucleotide toward the 3' end of the gene compared to the original reading frame. This mutation type gives a reading frame identical to that of (-2) mutations, wherein the encoded polypeptides differ by one amino acid. (-3) and (+3) mutations are irrelevant according to the present invention, for they do not give rise to frameshift polypeptides. According to the present invention the polypeptides included within the set shall be selected as to cover the widest possible range of tumours. Thus a set comprises for example (-1) frameshift polypeptides and additionally (+1) frameshift polypeptides. Using more than one possible novel reading frame of the particular gene broadens the spectrum of target cells and thus may prevent escape of particular cells from being eliminated according to the present invention.

A further aspect influencing the choice of the member polypeptides included in a set of frameshift polypeptides according to the present invention is the involvement of the gene encoded for by the coding sequence containing the respective microsatellite in particular biochemical pathways. The term biochemical pathway is used with a rather broad meaning herein. Biochemical pathways as used within this document shall for example include signal transduction pathways, enzymatic pathways, metabolic pathways, the apoptosis pathway, DNA repair or polymerization pathways, the pathway of meiosis etc.. To broaden the spectrum of tumours to be addressed by the set of frameshift polypeptides, members of different pathways are included in the set.

In a preferred embodiment at least 5 different pathways, in a more preferred embodiment at least 4 different pathways and in the most preferred embodiment at least 3 different pathways are represented by the frameshift polypeptides in a set according to the present invention. For example the TGF $\beta$ RII as a member of a signal transduction pathway may be used in combination with the BAX gene as a member of the apoptosis pathway with additional other polypeptides associated with other pathways.

A final aspect influencing the choice of suitable frameshift polypeptides to be included in the set according to the present invention is the length of the novel (poly)peptide sequence arising from the mutation. The shift of the reading frame leads to novel stop codons. Thus the new peptides are not of the same length as the polypeptides naturally encoded by the particular gene. In most cases the new peptides are shorter or even significantly shorter than the original wild-type polypeptide. Frequently rather oligopeptides arise from the frameshift mutations. The fidelity of the immune system in recognizing "foreign" molecules reaches thus far, to identify even polypeptides, that differ from "own" polypeptides in only one single amino-acid mutation. The fragments, bound by the antigen presenting HLA molecules comprise about 12 amino acid residues. To enhance the fidelity of recognition of new polypeptides more than one single amino-acid difference should be present. A polypeptide comprising 3 consecutive amino acids differing from the wild-type amino acid sequence is reliably recognized as foreign by the immune system. This may be due to the increased probability of new amino acid combinations being present in different fragments produced by the antigen presenting machinery. Thus the frameshift polypeptides according to the present invention contain at least one new amino acid, not present in



the wild-type polypeptide, in a more preferred embodiment at least 2 new amino acids and in the most preferred embodiment at least 3 new amino acids.

According to the named parameters a basic set of frameshift polypeptides may be tailored, that is suitable to address a large variety of tumours and minimizes the danger of escape of single tumour cells from the therapy. Thus the probability of survival of tumour cells in an organism following immuno-therapy can be minimized and the rate of recurrence of the cancer can be reduced.

A basic set of frameshift polypeptides includes frameshift polypeptides, that do occur with a high mutation frequency in associated disorders. Additionally the polypeptides within the set are chosen to be involved in different biochemical pathways. The mutation types are chosen, that polypeptides of a minimal length of 3 amino acid residues is expressed from the mutated nucleic acid sequence. Furthermore different mutation types of one single microsatellite may be included in the set if applicable.

Examples of basic sets of frameshift polypeptides for use in therapeutic as well as diagnostic methods of the present invention include:

Set1:	HT001	U79260	MACS	
Set2:	HT001	TAF1B	MACS	
Set3:	HT001	TGFB2R	MACS	
Set4:	HT001	U79260	TGFB2R	
Set5:	HT001	U79260	TAF1B	
Set6:	HT001	TGFB2R	TAF1B	
Set7:	HT001	U79260	TGFB2R	MACS
Set8:	HT001	U79260	TGFB2R	AC1
Set9:	HT001	U79260	TGFB2R	TAF1B
Set10:	HT001	TGFB2R	MACS	CASP5
Set11:	HT001	U79260	MACS	CASP5
Set12:	HT001	U79260	MACS	AC1
Set13:	HT001	TGFB2R	TAF1B	CASP5
Set14:	HT001	U79260	MACS	OGT
Set15:	U79260	TGFB2R	AC1	CASP5
Set16:	HT001	U79260	TGFB2R	MACS AC1
Set17:	HT001	U79260	TGFB2R	TAF1B MACS

				17	
Set18:	HT001	U79260	TGFB2R	TAF1B	AC1
Set19:	HT001	U79260	TGFB2R	MACS	AIM2
Set20:	HT001	U79260	TGFB2R	TAF1B	AIM2
Set21:	U79260	TGFB2R	TAF1B	AC1	CASP5
Set1:	HT001	U79260	TGFB2R	AC1	CASP5
Set22:	U79260	TGFB2R	MACS	AC1	CASP5
Set23:	HT001	U79260	TAF1B	MACS	AC1
Set24:	HT001	U79260	TAF1B	MACS	CASP5
Set1:	HT001	U79260	MACS	AC1	OGT
Set25:	HT001	U79260	MACS	MSH3	OGT
Set26:	HT001	U79260	TGFB2R	MACS	OGT
Set27:	HT001	TGFB2R	TAF1B	AC1	CASP5
Set28:	HT001	U79260	TGFB2R	AC1	AIM2

Additional to the parameters given above, data concerning the particular disorder in focus are taken into account for the design of particular sets of frameshift peptides according to the present invention. Thus individual mutation frequencies, typical  
5 mutation types, relevant biochemical pathways or special immunological characteristics may contribute to the set to be used in particular cases. Furthermore in particular cases based on results of examination of particular samples of individuals vaccines may be tailored as to optimally fit the therapy of the respective disorder. In one embodiment of the present invention individual tumour vaccine compositions may  
10 be set up according to molecular profiling of individual tumours

Choosing suitable combinations of the peptides within the mixture of frameshift peptides thus enables for generation of therapeutic or diagnostic preparations with a wide range of applicability. E.g. vaccines that elicit immune response specifically for tumours of particular organs. On the other hand it is possible to design sets of  
15 frameshift polypeptides that cover a wide range of degenerative disorders or cancers in individuals. The first possibility may e.g. be especially useful for the design of curative treatment, whereas the second variant of sets may be of special interest for the design of preventive vaccines.

The compositions and methods according to the present invention may be applied to  
20 any eukaryotic organisms exhibiting an immunologic defense system. The eukaryotic

organisms are for example animals of agricultural value such as pigs, cows, sheep, etc., companion animals, such as cats, dogs, horses etc., animals employed in research purposes such as mice, rats, rabbits, hamsters etc. or humans.

Therapeutic methods for use in the present invention comprise immunogenic treatment  
5 such as vaccination therapy or generally immuno-therapy. The vaccines for use in the present invention comprise for example one or more sets of frameshift polypeptides.

- According to the present invention frameshift polypeptides that comprise an immunogenic portion may be used for immuno-therapy for the treatment of cancer. Immunotherapy may be broadly classified into either active or passive immunotherapy.
- 10 In active immunotherapy, treatment relies on the in vivo stimulation of the endogenous host immune system to react against tumours with the administration of immune response-modifying agents (for example, tumour vaccines, bacterial adjuvants, and/or cytokines). A patient may be afflicted with disease, or may be free of detectable disease. Accordingly, the compounds disclosed herein may be used to treat cancer or  
15 to inhibit the development of cancer. The compounds are preferably administered either prior to or following primary treatment of tumours such as surgical removal of the tumours, treatment by administration of radiotherapy and/or conventional chemotherapeutic drugs or any other mode of treatment of the respective cancer or its precursors.
- 20 In passive immunotherapy, treatment involves the delivery of biologic reagents with established tumour-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumour effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T lymphocytes (for example, CD8+ cytotoxic T-lymphocytes, CD4+ T-helper, tumour-infiltrating  
25 lymphocytes), killer cells (such as Natural Killer cells, lymphokine-activated killer cells), B cells, or antigen presenting cells (such as dendritic cells and macrophages) expressing the disclosed antigens. The polypeptides disclosed herein may also be used to generate antibodies or anti-idiotypic antibodies (as in U.S. Pat. No. 4,918,164), for passive immunotherapy.
- 30 The predominant method of procuring adequate numbers of T-cells for adoptive transfer immunotherapy is to grow immune T-cells in vitro. Culture conditions for expanding single antigen-specific T-cells to several billion in number with retention of antigen recognition in vivo are well known in the art. These in vitro culture conditions

typically utilize intermittent stimulation with antigen, often in the presence of cytokines, such as IL-2, and non-dividing feeder cells. As noted above, the immunoreactive polypeptides described herein may be used to rapidly expand antigen-specific T-cell cultures in order to generate sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic-, macrophage- or B-cells, may be pulsed with immunoreactive polypeptides or transfected with a nucleic acid sequence(s), using standard techniques well known in the art. For example, antigen presenting cells may be transfected with a nucleic acid sequence, wherein said sequence contains a promoter region appropriate for increasing expression, and can be expressed as part of a recombinant virus or other expression system. For cultured T-cells to be effective in therapy, the cultured T-cells must be able to grow and distribute widely and to survive long term in vivo. Studies have demonstrated that cultured T-cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (see, for example, Cheever, M., et al, "Therapy With Cultured T-Cells: Principles Revisited," Immunological Reviews, 157:177, 1997).

According to the present invention sets of frameshift polypeptides may be employed to generate and/or isolate tumour-reactive T-cells, which can then be administered to the patient. In one technique, antigen-specific T-cell lines may be generated by in vivo immunization with short peptides corresponding to immunogenic portions of the disclosed polypeptides. The resulting antigen specific CD8+ CTL or CD4+ T-helper cells clones may be isolated from the patient, expanded using standard tissue culture techniques, and returned to the patient.

Alternatively, peptides corresponding to immunogenic portions of the polypeptides of the invention may be employed to generate tumour reactive T-cell subsets by selective in vitro stimulation and expansion of autologous T-cells to provide antigen-specific T-cells which may be subsequently transferred to the patient as described, for example, by Chang et al. (Crit. Rev. Oncol. Hematol., 22(3), 213, 1996). Cells of the immune system, such as T-cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as CellPro Incorporated's (Bothell, Wash.) CEPRATE.TM. system (see U.S. Pat. No. 5,240,856; U.S. Pat. No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a microsphere, to provide antigen-specific T-cells. The population of

tumour antigen-specific T-cells is then expanded using standard techniques and the cells are administered back to the patient.

In another embodiment, T-cell receptors and/or antibodies specific for the polypeptides can be cloned, expanded, and transferred into other vectors or effector cells for use in  
5 adoptive immunotherapy.

In a further embodiment, syngeneic or autologous dendritic cells may be pulsed with peptides corresponding to at least an immunogenic portion of a polypeptide disclosed herein. The resulting antigen-expressing and/or presenting dendritic cells may either be transferred into a patient, or employed to stimulate T-cells to provide antigen-specific T-cells which may, in turn, be administered to a patient. The use of peptide-pulsed dendritic cells to generate antigen-specific T-cells and the subsequent use of  
10 such antigen-specific T-cells to eradicate tumours in a murine model has been demonstrated by Cheever et al, (Immunological Reviews, 157:177, 1997).

Monoclonal antibodies directed against frameshift peptides presented on cellular  
15 membranes may according to the present invention also be used as therapeutic compounds in order to diminish or eliminate tumours. The antibodies may be used on their own (for instance, to inhibit metastases) or coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include <sup>90</sup>Y, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I, <sup>186</sup>Re, <sup>188</sup>Re, <sup>211</sup>At, and <sup>212</sup>Bi. Preferred drugs include methotrexate, and pyrimidine and purine analogues. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.  
20

Pharmaceutical compositions useful in immuno-therapy according to the present  
25 invention may comprise a set of at least 3, 4, 5, 6, 7, 8, 9 or 10 frameshift polypeptides (or variants thereof). Moreover sets of frameshift polypeptides may comprise 5 to 20, 7 to 30 or even more than 15, 20 or 30 frameshift peptides (or variants thereof). (or variants thereof). In certain embodiments the pharmaceutical compositions comprise the frameshift polypeptides and a physiologically acceptable carrier. The vaccines may  
30 additionally comprise a non-specific immune-response enhancer, wherein the non-specific immune response enhancer is capable of eliciting or enhancing an immune response to an exogenous antigen. Examples of non-specific-immune response enhancers include adjuvants, biodegradable microspheres (e.g., polylactic galactide)

and liposomes (into which the polypeptide is incorporated). Pharmaceutical compositions and vaccines may also contain other epitopes of tumour antigens, either incorporated into a fusion protein or present within a separate polypeptide.

Alternatively, a pharmaceutical composition or vaccine suitable for immunotherapy according to the present invention may contain nucleic acids, that code for one or more frameshift polypeptides according to the present invention. Nucleic acids may for example include single-stranded (sense or antisense) or double-stranded molecules, and may be DNA (genomic, cDNA or synthetic) or RNA. RNA molecules comprise as well HnRNA (containing introns) as mRNA (not containing introns). According to the present invention the polynucleotides may also be linked to any other molecules, such as support materials or detection marker molecules, and may, but need not, contain additional coding or non-coding sequences. The nucleic acid may be administered in a way that allows the polypeptides to be generated in situ. Suitable expression systems are known to those skilled in the art. The expression of the polypeptides may for example be persistent or transient. In pharmaceutical compositions and/or vaccines, providing for in-situ expression of polypeptides, the nucleic acids may be present within any suitable delivery system known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems.

Appropriate nucleic acid expression systems comprise the necessary regulatory nucleic acid sequences for expression in the patient, such as suitable promoters, terminators etc.. Bacterial delivery systems may for example employ the administration of a bacterium that expresses an epitope of a cell antigen on its cell surface. In a preferred embodiment, the nucleic acid may be introduced using a viral expression system such as e.g., vaccinia virus, retrovirus, or adenovirus, which may involve the use of a non-pathogenic, replication competent virus. Suitable systems are known to those of ordinary skill in the art and are disclosed, for example, in Fisher-Hoch et al., PNAS 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Pat. Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Pat. No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991; Kolls et al., PNAS 91:215-219, 1994; Kass-Eisler et al., PNAS 90:11498-11502, 1993; Guzman et al., Circulation 88:2838-2848, 1993; and Guzman et al., Cir. Res. 73:1202-1207, 1993.

In another embodiment transgenic mammalian cells may be used for delivery and/or expression of the nucleic acids. The methods for producing nucleic acid constructs suitable for in-situ expression of polypeptides are known to those of skill in the art.

Furthermore the nucleic acid may be administered as naked nucleic acids. In this case  
5 appropriate physical delivery systems, which enhance the uptake of nucleic acid may be employed, such as coating the nucleic acid onto biodegradable beads, which are efficiently transported into the cells. Administration of naked nucleic acids may for example be useful for the purpose of transient expression within a host or host cell.

The pharmaceutical compositions used for immuno-therapy according to the present  
10 invention may be administered by any suitable way known to those of skill in the art. The administration may for example comprise injection, such as e.g., intra-cutaneous, intramuscular, intravenous or subcutaneous injection, intranasal administration for example by aspiration or oral administration. A suitable dosage to ensure the pharmaceutical benefit of the treatment should be chosen according to the parameters,  
15 such as age, sex, body weight etc. of the patient, known to those of skill in the art.

The type of carrier to be employed in the pharmaceutical compositions of this invention, will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a lipid, a wax and/or a buffer. For oral administration, any of the above  
20 carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and/or magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic glycolide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Pat. Nos. 4,897,268  
25 and 5,075,109. Any of a variety of immune-response enhancers may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, Bordetella pertussis or Mycobacterium tuberculosis. Such  
30 adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.).

The pharmaceutical compositions or immuno-therapeutic methods according to the present invention may be used for the treatment of degenerative disorders or cancers. For example the compositions and methods may be employed in the therapy of diagnosed cancers in order to eliminate the tumour cells from the affected organism.

- 5 As well primary tumours as metastases or disseminated tumour cells within an organism may be targets to the therapeutic compounds and methods disclosed herein.

Furthermore the compositions and methods of the invention may be employed in the treatment of pre-neoplastic conditions. In this case the pre-neoplastic cells or tissues may be directly addressed by the immuno-therapeutic compositions or methods, or  
10 may be hindered from evolving into neoplastic or dysplastic conditions. For example in this case the pre-neoplastic condition may be treated preventively. By the vaccination the immune response may be elicited, so that emerging neoplasms may be destroyed.

The methods and compositions according to the present invention may also be used for the prevention of degenerative disorders or cancers associated with frameshift  
15 mutations in coding microsatellites. For this purpose a vaccination of a population of organisms or of subgroups of said population may be performed. The subgroups may be built by suitable parameters such as hereditary predisposition for the emergence of degenerative disorders, exposure to factors, that increase the risk of being affected by said disorders etc.

- 20 In one embodiment of the present invention the peptides disclosed herein may be used for the diagnosis of disorders associated with frameshift mutations in coding microsatellite regions.

Diagnosis as used in the context of the present invention may comprise determining the presence or absence and/or the level of frameshift peptides or of specific  
25 immunological entities directed against particular frameshift peptides in a sample, and assessing diagnosis from said presence or absence and/or level of frameshift peptides and/or immunological entities specifically directed against said frameshift polypeptides.

Based upon the determined presence or absence and/or the levels of frameshift peptides or of immunological entities specifically directed against particular frameshift  
30 peptides in the samples individuals can be subdivided into subgroups. The subgroups may be created according to scientific or clinical data, such as e.g. survival, recurrence of disease, frequency of metastases etc., related to the presence or absence and/or levels of frameshift peptides or of particular frameshift peptides in samples of tissues



affected with a particular disorder, of tissues being in question of being affected with a particular disorder or of tissues at risk of being affected with a particular disorder.

Based upon these subgroups an assessment of prognosis may be done. According to the subgroups the therapy of the individuals affected by the disorders (e.g. tumours) may be tailored.

Monitoring may comprise detecting the presence or absence or level of frameshift peptides or of immunologic entities specifically directed against frameshift polypeptides in samples taken at different points in time and determining the changes in said levels or presences or absences. According to said changes the course of the disease can be followed. E.g. the occurrence of frameshift peptides or of immunologic entities directed against frameshift peptides, that have not been present at an earlier time-point may be indicative of the progression of evolution of the affected tissue. The course of the disease may be used to select therapy strategies for the particular individual.

Another aspect of diagnosis and monitoring of the disease course according to the present invention may comprise the detection of minimal residual disease. This may comprise for example the detection of presence and/or level of frameshift peptides or of immunologic entities specifically directed against said frameshift polypeptides, that have not been present in earlier examinations in one or more body samples following initial therapy of an individual once or at several timepoints. According to the presence and/or level of frameshift peptides or of immunologic entities specifically directed against new frameshift polypeptides detected in the samples one may select a suitable therapy for the particular individual.

Furthermore the diagnostic method may be carried out to detect disseminated tumor cells in biological samples as MRD diagnosis of minimal residual disease. For this purpose the detection of the level of immunological entities or the presence of frameshift peptides or of immunological entities, specific for particular frameshift peptides, that have not been detected in prior examinations, may be performed.

Immunological entities as used in the context of the present invention shall comprise any components of the mammalian immune system, that are able to specifically react with an antigenic epitope. Such immunological entities may comprise for example antibodies, all immunoglobulins, such as e.g. IgG, IgM, IgA, IgE, IgD, specific CD8+ T-cells or specific T-helper cells.

A sample according to the method of the present invention may comprise any sample comprising frameshift peptides or immunological entities as defined above. Samples may comprise samples of clinical relevance, such as e.g. secretions, smears, body fluids, urine, semen, stool, bile, biopsies, cell- and tissue-samples. Biopsies as used in the context of the present invention may comprise e.g. resection samples of tumors, tissue samples prepared by endoscopic means or needle biopsies of organs.

Such samples may comprise for example intact cells, lysed cells or any liquids containing polypeptides, antibodies, immunoglobulins or cells specifically directed against frameshift peptides. Even solids, to which peptides, cells, cell fragments or antigen binding polypeptides, such as antibodies or immunoglobulins may adhere, or may be fixed to, may be samples according to the method disclosed herein. The method for detection of the level of the frameshift peptides or of the immunological entities according to the present invention is any method, which is suited to detect very small amounts of specific frameshift peptides or of specific immunological entities in biological samples. The detection reaction according to the present invention is a detection either on the level of polypeptides, nucleic acids, antibodies or on the level of cells specific for particular antigens.

For diagnostic purposes detection procedures related to one single frameshift polypeptides or to immunological entities specifically recognizing said frameshift peptides may be performed. Furthermore detection procedures may be performed that are tailored to display the presence or absence or the level of one or more sets of polypeptides or immunological entities directed against these polypeptides, wherein the sets have been put together according to rational combinatorial parameters as they are given below.

The detection may be carried out in solution or using reagents fixed to a solid phase. Solid phases may comprise beads of a variety of materials, such as e.g. agarose, dextrane polymers, polystyrene, silica, etc. or surfaces of suitable materials such as e.g. polystyrene, glass, agarose, protein, dextran etc. coated surfaces etc.. The detection of one or more immunological entities, such as immunoglobulins or cells carrying specific antigen recognizing epitopes, with different antigen binding specificities may be performed in a single reaction mixture or in two or more separate reaction mixtures. Alternatively the detection reactions for several immunological entities may for example be performed simultaneously in multi-well reaction vessels.

Applicable formats for the detection reaction according to the present invention may be, (reverse) blotting techniques, such as Western-Blot. The blotting techniques are known to those of ordinary skill in the art and may be performed for example as electro-blots, semidry-blots, vacuum-blots or dot-blots. Furthermore immunological methods for detection of molecules may be applied, such as for example immunoprecipitation or immunological assays, such as ELISA, RIA, Elispot assay, lateral flow assays, immuno-cytochemical methods etc..

The immunological entities specifically recognizing particular frameshift peptides may be detected using reagents that specifically recognise these immunological entities alone or in complex with their respective antigen (e.g. antibodies), or reagents, that are specifically recognized by the immunological entities themselves (e.g. the antigen). In one embodiment the antigen may be fused to another polypeptide, so as to allow binding of the antigen by the immunological entity in question and simultaneously binding of the second part of the fusion protein by another (labelled) antibody for the detection. The detection reaction for the immunological entities may comprise one or more reactions with detecting agents either recognizing the initial entities or recognizing the prior molecules used to recognize the immunological entities.

The detection reaction further may comprise a reporter reaction indicating the presence or absence and/or the level of the immunological entities. The reporter reaction may be for example a reaction producing a coloured compound, a bioluminescence reaction, a chemiluminescent reaction, a fluorescence reaction, generally a radiation emitting reaction etc.. The detection reactions may for example employ antibodies or binding reagents that are detectably labelled.

Furthermore the binding of detection molecules to the entities in question may be detected by any measurable changes in physical or physical-chemical properties, such as changes in spectroscopic properties, in magnetic resonance properties etc.. Different immunological entities or immunological entities of different specificities may be recognized by different methods or agents. This may be due to difficulties in detection of several entities, or entities with particular specificities by a particular method. An advantage of the use of different detection techniques for different immunological entities or for immunological entities with different specificities may for example be, that the different reporter signals referring to different immunological entities could be distinguished.

Generally in a method according to the present invention the detection of different immunological entities such as the detection of immunoglobulins and the detection of immunocompetent cells may be performed simultaneously.

For all detection purposes optionally the original sample may be concentrated by any suitable means known to those of ordinary skill in the art. Furthermore steps may be involved to selectively extract immunological entities from the sample mixture such as affinity based purification techniques either employing specific antibodies or the respective antigen recognized by the entities in question.

In one preferred embodiment of the invention the detection of the level of immunological entities specific for frameshift peptides is carried out on the level of antibodies. This may be e.g. performed using the specific interaction between the respective frameshift peptides with the antibodies. The determination of the presence or absence and/or the level of the antibodies may for example be carried out with recombinantly produced frameshift peptides. The peptides can be used in many different detection techniques for example in western-blot, ELISA or immunoprecipitation. In one embodiment the detection of antibodies is carried out as antibody capture assay (Antibodies A laboratory Manual, Harlow, Ed. et al., Cold Spring Harbor Laboratory 1988).

In another embodiment of the invention the detection of the specific antibodies is carried out using monoclonal or polyclonal antibodies specifically recognizing the antigen binding epitope of the first antibodies. For this purpose the above mentioned immunological detection procedures may be applied. In a further embodiment chimeric antigens may be employed in the detection reaction. Such chimeric antigens may for example comprise fusion proteins combining the antigenic epitope of a frameshift polypeptide, recognized by the antibody in question, fused to another antigen, that may be recognized by a detection antibody. The particular antigens within the chimeric polypeptide may be separated by a linker or spacer region.

Any other method for determining the amount of particular antibodies or immunoglobulins in biological samples can be used according to the present invention.

Generally the detection of the antibodies according to the present invention may be performed as well in vitro as directly in situ for example in the course of an immunohistochemical or immuno-cytochemical staining reaction.

Cells exhibiting specificity for a particular antigen may be detected by any methods suitable for that purpose known to those of ordinary skill in the art. Methods may for example comprise proliferation-assays, cytokine-ELISAs, ELISpot assays, intracellular FACS-staining, PCR-mediated identification of peptide-specific cytokine (or similar) -  
5 expressing cells, tetramer-staining, cytotoxicity assays and DTH-(delayed type hypersensitivity) reactions.

In case of proliferation-assays induction of peptide-specific T-cell proliferation may be measured by methods known to those of skill in the art. This can be achieved by simply counting of cells, by measuring incorporation of labelled nucleotides into cellular  
10 DNA or by measuring level and/or activity of cellular protein(s). Cytokine-ELISA may comprise identification of peptide-specific cytokine-secreting cells by measuring cytokine levels in supernatant. In the course of an ELISpot assay the number of peptide-specific cytokine (i.e. IFN-g)- secreting cells in a sample is determined. Similarly the Intracellular FACS-staining identifies cytokine-expressing cells on the  
15 protein level. In contrast (real-time) PCR may be used for identification of peptide-specific cytokine (or similar) -expressing cells on the transcript level. In the course of a tetramer-staining assay the label is a tetramer-molecule of recombinant MHC-class I molecules, loaded with specific peptide and coupled to a dye. The tetramer binds to the T-cell receptor. Cytotoxicity assays are a method for identification of cells, that can  
20 recognize and kill target cells in a peptide-specific manner. DTH-(delayed type hypersensitivity) reaction is based on the measuring of skin-reaction of vaccinated persons after intradermal (or similar) application of peptide(s).

In a preferred embodiment of the invention the detection of the immunological entities directed against particular frameshift polypeptides is carried out on the level of  
25 antibodies. In this embodiment the binding agent may be for example a frameshift polypeptide or a fragment thereof, recognized by the respective antibody, or a fusion polypeptide comprising said frameshift polypeptide or a fragment thereof. Furthermore the binding agent may comprise an antibody or a fragment thereof specific for the antibody in question, for the complex of the antibody with the respective frameshift  
30 polypeptide or for an antigenic epitope fused to the frameshift polypeptide.

In another embodiment of the test kit the detection of the immunological entities is carried out on the level of cells specifically recognizing frameshift polypeptides. In this embodiment of the invention the reagent for the detection may be for example a

frameshift polypeptide or a fragment thereof, recognized by the respective antibody or T-cell receptor, or a fusion polypeptide comprising said frameshift polypeptide or a fragment thereof. Furthermore the binding agent may comprise an antibody or a fragment thereof specific for the antibody in question, for the complex of the antibody  
5 with the respective frameshift polypeptide or for an antigenic epitope fused to the frameshift polypeptide.

The method for detection of the level of the frameshift polypeptides according to the present invention is any method, which is suited to detect very small amounts of specific biologically active molecules in biological samples. The detection reaction  
10 according to the present invention is a detection either on the level of nucleic acids or on the level of polypeptides.

The detection may be carried out in solution or using reagents fixed to a solid phase. The detection of one or more molecular markers, such as polypeptides or nucleic acids, may be performed in a single reaction mixture or in two or separate reaction  
15 mixtures. Alternatively the detection reactions for several marker molecules may for example be performed simultaneously in multi-well reaction vessels. The markers characteristic for the frameshift polypeptides may be detected using reagents that specifically recognise these molecules. The detection reaction for the marker molecules may comprise one or more reactions with detecting agents either  
20 recognizing the initial marker molecules or recognizing the prior molecules used to recognize other molecules.

In one preferred embodiment of the invention the detection of the level of frameshift polypeptides is carried out by detection of the level of nucleic acids coding for the frameshift polypeptides or fragments thereof present in the sample. The means for  
25 detection of nucleic acid molecules are known to those skilled in the art. The procedure for the detection of nucleic acids can for example be carried out by a binding reaction of the molecule to be detected to complementary nucleic acid probes, proteins with binding specificity for the nucleic acids or any other entities specifically recognizing and binding to said nucleic acids. This method can be performed as well in vitro as directly  
30 in situ for example in the course of a detecting staining reaction. The use of this detection procedure is restricted to cases, where hybridisation properties of the respective frameshift mutations are significantly altered in comparison to the respective wild-type nucleic acids. Another way of detecting the frameshift polypeptides in a

sample on the level of nucleic acids performed in the method according to the present invention may comprise an amplification reaction of nucleic acids. In these cases a subsequent reaction displaying the presence or absence of a frameshift mutation within the coding microsatellite region is necessary.

- 5 In another preferred embodiment of the invention the detection of the level of frameshift polypeptides is carried out by determining the level of expression of a protein. The determination of the frameshift polypeptides on the protein level can for example be carried out in a reaction comprising an antibody specific for the detection of the frameshift polypeptides. The antibodies can be used in many different detection
- 10 techniques for example in western-blot, ELISA or immuno-precipitation. Generally antibody based detection can be carried out as well in vitro as directly in situ for example in the course of an immuno-histochemical staining reaction. Any other method for determining the amount of particular polypeptides in biological samples can be used according to the present invention.
- 15 Furthermore according to the present invention diagnosis may comprise detection of immunological entities specifically recognizing particular frameshift peptides using reagents that specifically recognise these immunological entities alone or in complex with their respective antigen (e.g. antibodies), or reagents, that are specifically recognized by the immunological entities themselves (e.g. the antigen). In one
- 20 embodiment the antigen may be fused to another polypeptide, so as to allow binding of the antigen by the immunological entity in question and simultaneously binding of the second part of the fusion protein by another (labelled) antibody for the detection. The detection reaction for the immunological entities may comprise one or more reactions with detecting agents either recognizing the initial entities or recognizing the prior
- 25 molecules used to recognize the immunological entities.

The detection reaction further may comprise a reporter reaction indicating the presence or absence and/or the level of the frameshift polypeptides or of the immunological entities. The reporter reaction may be for example a reaction producing a coloured compound, a bioluminescence reaction, a chemiluminescent reaction, a

30 fluorescence reaction, generally a radiation emitting reaction etc.. The detection reactions may for example employ antibodies or binding reagents that are detectably labelled.

Furthermore the binding of detection molecules to the peptides or immunological entities in question may be detected by any measurable changes in physical or physical-chemical properties, such as changes in spectroscopic properties, in magnetic resonance properties etc.. Different polypeptides or immunological entities or immunological entities of different specificities may be recognized by different methods or agents. This may be due to difficulties in detection of several entities, or entities with particular specificities by a particular method. An advantage of the use of different detection techniques for different polypeptides and/or immunological entities or for immunological entities with different specificities may for example be, that the different reporter signals referring to different immunological entities could be distinguished.

Generally in a method according to the present invention the detection of different polypeptides, of different immunological entities such as the detection of immunoglobulins and the detection of immunocompetent cells may be performed simultaneously.

For all detection purposes optionally the original sample may be concentrated by any suitable means known to those of ordinary skill in the art. Furthermore steps may be involved to selectively extract polypeptides and/or immunological entities from the sample mixture such as affinity based purification techniques either employing specific antibodies or the respective antigen recognized by the entities in question.

Another aspect of the present invention is a testing kit for performing the method according to the present invention. The kit may be for example a diagnostic kit or a research kit.

A kit according to the present invention comprises at least an agent suitable for detecting the immunological entities according to the method disclosed herein.

Furthermore a kit according to present invention may comprise:

- a) reagents for the detection of the antibodies or cells specifically recognizing antigens.
- b) reagents and buffers commonly used for carrying out the detection reactions as described herein, such as buffers, detection-markers, carrier substances and others
- c) a sample for carrying out a positive control reaction, that may comprise an antigen or a set of antigens, to which all members of a target population of individuals have antibodies.



The reagent for the detection of the antibodies or cells specifically recognizing antigens may include any agent capable of binding to the antibodies or cells specifically recognizing antigens. Such reagents may include proteins, polypeptides, nucleic acids, peptide nucleic acids, glycoproteins, proteoglycans, polysaccharids or lipids.

- 5 The sample for carrying out a positive control may comprise for example an antigenic peptide or a set of antigenic peptides. Suitable antigens may include antigens, against which a wide percentage of the population has antibodies. Examples of such antigens may for example comprise antigens present in lysed E. coli cells, tetanus antigen, the viral capsid antigen of the Epstein-Barr virus, antigens derived from matrix proteins of
- 10 Haemophilus influenzae. The antigens may for example be used as a mixture to ensure, that a particular individual actually displays a positive reaction.

The present invention provides compositions and methods for enhanced immunotherapy of disorders associated with MSI<sup>+</sup> related occurrence of frameshift peptides. The invention provides sets for the immuno-therapy of said disorders, that

15 address a wide range of different types of disorders in an organism and may thus be employed as a preventive vaccine against said disorders. Furthermore the invention also provides sets of polypeptides useful for the treatment of particular types of disorders. The use of sets of frameshift polypeptides for the immuno-therapy according to the present invention provides the means for a reliable treatment of degenerative

20 disorders and cancers associated with frameshift mutations in coding microsatellites reducing the risk of escape of several tumour cells or of a population of tumour cells from being addressed by the therapy. Thus the method of the invention reduces the risk of recurrence of tumours after immuno-therapy employing CTLs, T-helper cells and possibly specific antibody producing B-cells raised against frameshift polypeptides

25 characteristic for tumour cells. The present invention furthermore provides compositions and methods for enhanced diagnosis and therapy of disorders associated with MSI<sup>+</sup> related occurrence of frameshift peptides. The invention provides new frameshift polypeptides for diagnosis therapy of said disorders, that may be used each alone or in combinations tailored, to address a wide range of different types of disorders in an

30 organism and may thus be employed as a preventive vaccine against said disorders.

Brief description of the drawings:

Figure 1: **ELISpot-analysis of FSP T-cell lines.** Titrated amounts of T-cells were incubated overnight with  $3.5 \times 10^4$  peptide loaded T2 cells per well. The number of IFN- $\gamma$ -releasing activated T-cells (spots) among the total number of cells analyzed ( $10^6$ ) is depicted for each frameshift peptide. Reactivity against peptide YLLPAIVHI from the nuclear protein P68 served as a negative control and is indicated (open bars).

Figure 2: **Listing of sequences of polypeptides encoded by genes with coding microsatellites;** the sequences of polypeptides arising from different possible frameshift mutations are depicted. For each polypeptide the sequence expressed from the wild type open reading frames is given (wtORF); Furthermore the sequences expressed from (-1) mutations and from (-2/+1) mutation are given.

Figure 3: **Frameshift peptide specific and HLA-A2-restricted lysis of target cells. (FSP02);** The antigen specificity of the FSP02 CTL line was tested in the presence of unlabeled cold targets, T2 cells pulsed with FSP02 (open squares) at an inhibitor:target ratio of 50:1. Lysis without cold targets is shown as a control (closed squares). All data are shown as the mean and standard deviation from 3 replicate wells. For experimental details see example 6.

Figure 4: **Frameshift peptide specific and HLA-A2-restricted lysis of target cells. (FSP06);** The antigen specificity of the FSP06 CTL line was tested in the presence of unlabeled cold targets, T2 cells pulsed either with FSP06 (open squares) at an inhibitor:target ratio of 50:1. Lysis without cold targets is shown as a control (closed squares). All data are shown as the mean and standard deviation from 3 replicate wells. For experimental details see example 6.

The following examples are given for illustration of the invention only and are not intended to limit the scope of the invention.

Example 1: **Analysis of the mutation frequency of genes harbouring repeat tracts**

Investigations were performed regarding the mutation frequencies of unpublished

coding microsatellite regions. Nine novel coding microsatellites residing in genes not yet analyzed for frameshift mutations in MSI colorectal, endometrial or gastric tumours have been examined in the course of the studies leading to the present invention. They include three genes containing A11 repeats (TAF1B, MACS, HT001), five genes with  
5 A10 repeats (CHD2, UVRAG, TCF6L1, ABCF1, AIM2) and one gene harboring a G9 repeat (ELAVL3). The MSI status of these specimen was determined using the NCI ICG-HNPCC microsatellite reference marker panel (8). PCR reactions were performed as follows:

Genomic DNA was isolated from 5-8 haematoxylin and eosin stained 5  $\mu$ m sections  
10 after microdissection, using the Qiamp Tissue Kit (Qiagen, Hilden, Germany). Preparation of DNA from cell lines was performed according to standard protocols. PCR primers were designed to closely flank the target sequence, yielding short amplicones of about 100 base pairs thus allowing precise fragment sizing and robust amplification from archival tissues (Table 1). PCR reactions were performed in a total  
15 volume of 25  $\mu$ l containing 50 ng genomic DNA, 2.5  $\mu$ l 10 x reaction buffer (Life Technologies, Karlsruhe, Germany), 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs, 0.3  $\mu$ M of each primer, and 0.5 U Taq DNA polymerase (Life Technologies) and using the following conditions: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, and primer extension at 72 °C for 30 s.  
20 The final extension step was carried out at 72 °C for 6 min. PCR fragments were analyzed on an ALF DNA sequencing device (Amersham Pharmacia Biotech, Freiburg, Germany) using 6.6 % polyacrylamide/ 7 M urea gels. Size, height and profile of microsatellite peaks were analyzed using the AlleleLinks software (Amersham Pharmacia Biotech). Coding microsatellite instability was scored, if smaller  
25 or larger-sized amplicones were detected in tumour DNA compared to DNA from non-neoplastic cells. Allele intensities were determined and ratios of wild-type and novel alleles in normal and tumour tissues were calculated, defining a two-fold difference as threshold for allelic shifts. Similarly, unstable alleles in tumour cell lines were identified by comparison to 36 unmatched normal mucosae. In order to determine the predicted  
30 repeat type and length amplified coding microsatellites were subjected to Big Dye terminator cycle sequencing (Perkin Elmer, Darmstadt, Germany) and subsequent analysis on an ABI 310 sequencing device.

The frequencies of mutations in a particular microsatellite region examined herein are given in figure 1/2. The mutation rates are calculated with respect to the total number of samples included in the study.

**Table 1: Coding microsatellite genes Investigated in MSI-H colorectal cancer.**

5 Repeat: type of nucleotide and length of repeat tract, n: number of samples investigated, mut.: number of samples mutated, %: percentage of mutated samples,

gene	acc.no.	repe at	n	mut.	%
HPDMPK	Y10936	T14	21	20	95%
HT001	AF1135	A11	20	17	85%
	39				
IGF1R	D50633	A10	735	602	82%
U79260	U79260	T14	21	17	81%
PTHL3	M2435	A11	21	17	81%
	0				
MACS	D10522	A11	19	14	74%
TAF1B	L39061	A11	19	13	68%
AC1	D82070	T10	21	14	67%
AIM2	AF0247	A10	19	10	53%
	14				
PAX5	U22473	G8	588	265	44%
SLC23A1	AF0583	C9	21	9	43%
	19				
ABCF1	AF0273	A10	19	8	42%
	02				
TCF-4	Y11306	A9	248	98	40%
Caspase 5	U28015	A10	120	47	39%
TCF6L1	M8507	A10	18	7	39%
	9				
FLT3LG	U29874	C9	21	8	38%
MSH3	J04810	A8	596	223	37%
ELAVL3	D26158	G9	19	7	37%
MAC30X	L19183	A10	21	7	33%
UVRAG	X99050	A10	18	6	33%
SLC4A3	U05596	C9	21	7	33%
GRB-14	L76687	A9	57	18	32%
RIZ	U17838	A9	83	23	28%
MBD4/MED1	AF0722	A10	83	22	27%
	50				
RAD50	U63139	A9	109	28	26%
MSH6	U54777	C8	684	169	25%
IGF1R	Y00285	G8	423	90	21%
Axin2	AF2058	G7	45	9	20%
	88				
GART	X54199	A10	21	4	19%
Bcl-10	AF0822	A8	32	6	19%

	83				
RHAMM	U29343	A9	57	9	16%
PTEN	U92436	A6	32	5	16%
OGT	U77413	T10	78	10	13%
BLM	U39817	A9	170	19	11%
Fas	X63717	T7	30	3	10%
Apaf-1	AF0132	A8	43	4	9%
	63				
MLH3	AF1956	A9	99	9	9%
	57				
CBF	M3719	A9	57	5	9%
	7				
HTP1	AB024	A9	57	5	9%
	582				
RECQL	L36140	A9	68	5	7%
RBBP8	U72066	A9	70	5	7%
MLH3	AF1956	A8	123	7	6%
	57				
PTEN	U92436	A6	32	2	6%
CHD2	AF0065	A10	19	1	5%
	14				
ATRmRNA	U76308	A10	77	4	5%
INPPL1/DRP	L24444	C7	82	4	5%
CHK1	AF0165	A9	21	1	5%
	82				
SYCP1	X95654	A10	71	3	4%
RIZ	U17838	A8	83	3	4%
ANG2	AF0043	A9	57	2	4%
	27				
KKIAMRE/C	U35146	A9	57	2	4%
DKL2					
ATM	U82828	T7	39	1	3%
CDX2	Y13709	G7	45	1	2%
Axin2	AF2058	A6	45	1	2%
	88				
BRCA1	U14680	A8	92	2	2%
Doc-1	U53445	A9	57	1	2%
BRCA2	U43746	A8	119	2	2%
RFC3	L07541	A10	76	1	1%
Casp8AP2/F	AF1544	A9	13	0	0%
LASH	15				
ERCC5/XPG	D16305	A9	13	0	0%
HUMGPRKL	L03718	A9	57	0	0%
G					
DP2	U18422	A9	57	0	0%
PMS2	U14658	A8	133	0	0%
Caspase 1/I	M8750	A8	42	0	0%
CE	7				
WRN	L76937	A8	11	0	0%
POLA	X06745	A8	83	0	0%
NSEP	M8523	C8	132	0	0%

	4				
SHC1	U73377	G8	11	0	0%
NBS1	AF0586	A7	39	0	0%
	96				
BRCA2	X95152	T6	31	0	0%

Table 2: Coding microsatellite genes investigated in MSI-H gastric cancer.

Repeat: type of nucleotide and length of repeat tract, n: number of samples investigated, mut.: number of samples mutated, %: percentage of mutated samples

gene	acc.no.	repe at	n	mut.	%
HPDMPK	Y10936	T14	15	15	100%
TA1B	L3906	A11	15	13	87%
PTHL3	M2435	A11	15	11	73%
	0				
MACS	D10522	A11	15	9	60%
TGFbIIIR	M8507	A10	227	122	54%
	9				
HT001	AF1135	A11	15	8	53%
	39				
MBD4/MED1	AF0722	A10	15	7	47%
	50				
RIZ	U17838	A9	51	23	45%
Caspase 5	U28015	A10	25	11	44%
AIM2	AF0247	A10	15	6	40%
	14				
OGT	U77413	T10	15	6	40%
SLC23A1	AF0583	C9	15	6	40%
	19				
BAX	L22473	G8	208	72	35%
MSH6	U54777	C8	224	76	34%
ABCF1	AF0273	A10	15	5	33%
	02				
FLT3LG	U29874	C9	15	5	33%
MSH3	J04810	A8	202	65	32%
RAD50	Z75311	A9	36	10	28%
PRKDC	U63630	A10	30	8	27%
SLC4A3	U05596	C9	15	4	27%
BLM	U39817	A9	36	9	25%
ATRmRNA	U76308	A10	18	4	22%
B2M	AB021	A5	28	6	21%
	288				
MAC30X	L19183	A10	15	3	20%
RFC3	L07541	A10	15	3	20%
UVRAG	X99050	A10	15	3	20%
AC1	D82070	T10	15	3	20%
IGFIIR	Y00285	G8	202	36	18%
Apaf-1	AF0132	A8	20	3	15%

	63				
TCF-4	Y11306	A9	23	3	13%
SYCP1	X95654	A10	40	4	10%
Bcl-10	AF0822	A8	20	2	10%
	83				
Fas	X63717	T7	20	2	10%
U79260	U79260	T12	15	1	7%
GART	X54199	A10	15	1	7%
TCF6L1	M8507	A10	15	1	7%
	9				
ELAVL3	D26158	G9	15	1	7%
ATM	U82828	T7	36	2	6%
BRCA1	U14680	A8	64	3	5%
INPPL1/DRP	L24444	C7	25	1	4%
RIZ	U17838	A8	51	1	2%
CHD2	AF0065	A10	15	0	0%
	14				
CHK1	AF0165	A9	15	0	0%
	82				
BRCA2	U43746	A8	68	0	0%
PMS2	U14658	A8	30	0	0%
NSEP	M8523	C8	50	0	0%
	4				
NBS1	AF0586	A7	36	0	0%
	96				

The results presented in Tables 1 and 2 show, that the tested microsatellite regions are frequently mutated in tumour samples.

**Example 2: Detection of the expression of frameshift mutated mRNA from genes harbouring coding microsatellite regions using PCR**

Samples of colon, gastric and endometrial carcinomas are used to determine the expression of mRNA showing frameshift mutations in coding microsatellite regions using PCR and subsequent sequencing of the amplified nucleic acid products.

- 10 Tumours are collected, snap frozen, and stored at -80°C. They are verified to be composed predominantly of neoplastic cells by histopathological analysis. mRNA is isolated from tumours using Qiagen reagents (Qiagen, Hilden, Germany), and single-stranded cDNA is synthesized using Superscript II (Life Technologies, Inc.).

- 15 PCR reactions were performed in a total volume of 25 µl containing 50 ng cDNA, 2.5 µl 10 x reaction buffer (Life Technologies, Karlsruhe, Germany), 1.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 0.3 µM of each primer, and 0.5 U Taq DNA polymerase (Life Technologies)

and using the following conditions: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, and primer extension at 72 °C for 30 s. The final extension step was carried out at 72 °C for 6 min. PCR fragments were analyzed on an ALF DNA sequencing device (Amersham Pharmacia Biotech, Freiburg, Germany) using 6.6 % polyacrylamide/ 7 M urea gels.

The experiments described show, that in the cases of the tested genes CHD2, UVRAG, ELAVL3, TCF6L1, ABCF1, AIM2, TAF1B, MACS and HT001 mutated coding microsatellite regions are transcribed into mRNA.

These results indicate, that the cells harbouring mutations in coding microsatellite regions of the nine genes tested express neo-polypeptides derived from these frameshift mutations.

#### Example 3: Stimulation of cellular immune response by frameshift peptides

The present experiments were performed in order to determine, whether the frameshift peptides arising from mutations in coding microsatellite regions according to the present invention are suited to stimulate a cellular immune response. The experiments were performed as follows:

Peptides displaying HLA-A2.1-binding motifs were selected by taking advantage of specific computer programs [(Parker, Bednarek, & Coligan 1994); [http://bimas.dcrt.nih.gov/molbio/hla\\_bind/](http://bimas.dcrt.nih.gov/molbio/hla_bind/) and (Rammensee et al. 1999); <http://134.2.96.221/scripts/MHCServer.dll/home.htm>].



**Table 3: Frameshift Peptides analysed for in vitro stimulation of a cellular immune response;** All analysed peptides are derived from (-1) mutations in microsatellites of the cognate proteins; The protein or nucleotide accession numbers are indicated within the table; the position of the start amino acid in the protein is indicated in the tables; the predicted binding scores to HLA-A2.1 using computer assisted analysis;

Protein	Accession Number	Name	Peptide	Theoretical Scores	
				Ken Parker	SYFPEITHI
TGF-betaRII (-1)	AAA61164	FSP01	<sup>128</sup> -SLVRLSSCV	70	23
TGF-betaRII (-1)	AAA61164	FSP02	<sup>131</sup> -RLSSCPVA	5	19
TGF-betaRII (-1)	AAA61164	FSP03	<sup>135</sup> -CVPVALMSA	1	14
HPDMPK (-1)	CAA71862	FSP04	<sup>136</sup> -LLHSAPTPSL	36	25
HPDMPK (-1)	CAA71862	FSP05	<sup>129</sup> -FLSASHFLL	570	21
HPDMPK (-1)	CAA71862	FSP07	<sup>125</sup> -RVFFFYQHL	39	15
OGT (-1)	AAB63466	FSP06	<sup>128</sup> -SLYKFSFPFL	397	23
D070 (-1)	BAA11534	FSP08	<sup>35</sup> -KIFTFFFQL	1593	21
D070 (-1)	BAA11534	FSP09	<sup>68</sup> -ALLPAGPLT	28	21
D070 (-1)	BAA11534	FSP10	<sup>69</sup> -LLPAGPLTQT	29	20
U79260 (-1)	AAB50206	FSP11	<sup>59</sup> -TLSPGWSAV	118	25
U79260 (-1)	AAB50206	FSP12	<sup>83</sup> -ILLQPPEWL	362	26
Sec63 (-1)	AAC83375	FSP13	<sup>551</sup> -RQMESLGMKL	33	15
MAC30X (-1)	AAA16188	FSP14	<sup>188</sup> -VEMPTGWLL	20	14
MAC30X (-1)	AAA16188	FSP15	<sup>188</sup> -VEMPTGWLLV	14	15
FLT3L (-1)	U29874	FSP16	<sup>113</sup> -FQPPPAVFA	13	10
MSH-3 (-1)	AAB47281	FSP17	<sup>389</sup> -ALWECSLPQA	389	24
MSH-3 (-1)	AAB47281	FSP18	<sup>388</sup> -FLLALWECSL	364	25
MSH-3 (-1)	AAB47281	FSP19	<sup>387</sup> -LLALWECSL	36	26
MSH-3 (-1)	AAB47281	FSP20	<sup>394</sup> -SLPQARLCL	21	23
MSH-3 (-1)	AAB47281	FSP21	<sup>402</sup> -LIVSRTLLL	5	23
MSH-3 (-1)	AAB47281	FSP22	<sup>401</sup> -CLIVSRTLL	21	22
MSH-3 (-1)	AAB47281	FSP23	<sup>403</sup> -IVSRTLLLV	24	21
MSH-3 (-1)	AAB47281	FSP24	<sup>382</sup> -KRATFLAL	0,1	20
Caspase-5 (-1)	U28015	FSP25	<sup>61</sup> -KMFFMVFLI	1301	20
Caspase-5 (-1)	U28015	FSP26	<sup>67</sup> -FLIIWQNTM	22,85	21
TAF-1b (-1)	L39061	FSP27	<sup>108</sup> -GMCVKVSSI	17	24
HT001 (-1)	NP 054784	FSP30	<sup>281</sup> -VLRTGEPL	n.d.	21
MSH-3 (-1)	AAB47281	FSP31	<sup>402</sup> -LIVSRTLLLV	37	25
MSH-3 (-1)	AAB47281	FSP32	<sup>394</sup> -SLPQARLCLI	24	24

			41		
MSH-3 (-1)	AAB47281	FSP33	<sup>401</sup> CLIVSRTL	21	23
MSH-3 (-1)	AAB47281	FSP34	<sup>399</sup> RLCLIVSRTL	4	22

Peptides were purchased from the peptide synthesis unit of the DKFZ. Stock solutions (10mg/ml in DMSO) were stored at -70°C and diluted to 1mg/ml in PBS before use. T2 cells were pulsed with 50µg/ml peptide and 5µg/ml β2-microglobulin (Sigma; Deisenhofen, Germany) overnight at 37°C. The expression of HLA-A2.1 was then analysed by flow cytometry using mAb BB7.2 followed by incubation with FITC-conjugated (ab')2 goat anti-mouse Ig (Vonderheide et al. 1999).

Peripheral blood was obtained from a healthy HLA-A2.1+ donor and collected in heparinized tubes. PBMNC were isolated by Ficoll-density gradient centrifugation. Whole CD3+ T-cells were isolated from PBMNC by magnetic depletion of non-T-cells using the MACS Pan T-cell Isolation Kit (Miltenyi; Bergisch Gladbach, Germany) according to manufacturer's instructions. Preparations contained at least 97% of CD3+ cells as assessed by immunophenotypic analysis.

HLA-A2.1-restricted peptides were FSP27 and FSP29 from a (-1) in the TAF1B-gene; FSP30 was derived from a (-1) mutation in the HT001 gene.

CD40 Bs of a HLA-A2.1+ donor were incubated with peptide (10µg/ml) and human β2-microglobulin (3µg/ml; Sigma) in serum-free Iscov's DMEM medium for one hour at room temperature, washed twice to remove excess of peptide, were irradiated (30Gy) and added to purified CD3+ autologous T-cells (>97% CD3+) at a ratio of 4:1 (T:CD40 Bs) in Iscov's MEM containing 10% human AB-serum, supplements (1:100) and hIL-7 (10ng/ml, R&D). Cells were plated at a density of 2 x 10<sup>6</sup> T-cells/well in 1ml of medium. After three days in culture they were fed with 1ml complete medium. For restimulation of T-cells, this was repeated weekly. IL-2 was first given at day 21 (10IU/ml, R&D), also at day 24 and from day 28 on only hIL-2 was used instead of hIL7.

ELISpot assays were performed as described elsewhere (Meyer et al. 1998). Briefly, nitrocellulose-96-well plates (Multiscreen; Millipore, Bedford, USA) were covered with mouse anti-human IFN-γ monoclonal antibody (Mabtech, Sweden) and blocked with serum containing medium. Varying numbers of effector cells were plated in triplicates with 3,5x10<sup>4</sup> peptide-loaded T2 cells per well as targets. After incubation for 18h, plates were washed, incubated with biotinylated rabbit anti-human IFN-γ second antibody, washed again, incubated with streptavidin coupled alkaline phosphatase,

42  
followed by a final wash. Spots were detected by incubation with NBT/BCIP (Sigma) for 45min, reaction was stopped with water, after drying spots were counted using the KS-ELISpot reader (Zeiss Kontron; Göttingen, Germany).

The analysis shows, that the used peptides are suited to raise an immune response.

- 5 Peptides arising from frameshift mutations thus may be used to raise immune response for example in the course of vaccinations according to the present invention.

**Example 4: Screening for Antibodies directed against frameshift peptides in patient samples**

10

Serum of 25 patients with diagnosed colorectal carcinomas was tested for the presence of antibodies to a set of frameshift peptides arising from frameshift mutations in coding microsatellite regions of the following genes: CHD2, UVRAG, ELAVL3, TCF6L1, ABCF1, AIM2, TAF1B, MACS and HT001. As a control the serum of 50  
15 normal individuals was tested.

20

For the test synthetic peptides representing immunogenic portions of all relevant frameshift peptides (see figure 5) arising from the respective genes were spotted onto nylon membranes. The nylon membranes were subsequently incubated for one hour in phosphate-buffered saline (PBS) with 5% milk powder for blocking unspecific  
20 membrane binding. After washing the membranes 3x with PBS, the membranes were incubated with the test and control sera. The sera were diluted 1:1.000 in PBS/0,5% milk powder and incubated overnight with gentle shaking. Subsequently the sera were removed, and membranes were washed three times in PBS before they were incubated with a polyclonal alkaline phosphatase conjugated goat anti-human IgG antibody for  
25 one hour. Thereafter, the membranes were washed repeatedly with PBS/0,05% TWEEN20 before staining reaction was developed using nitroblue tetrazolium chloride and bromochloro-indoyl-phosphate (SigmaAldrich) in Tris-buffered saline (TBS). Binding of human antibodies specific for individual frameshift polypeptides thus was made visible by color-deposit on the respective membrane.

25

- 30 The results show, that in all samples of tumour patients antibodies directed against at least one peptide arising from frameshift mutations were present.

This illustrates, that the method according to the present invention may be used for diagnosis of diseases associated with frameshift mutations in coding regions of genes.

5                   **Example 5:     In vitro stimulation of cellular immune response by frameshift peptides**

10       The present experiments were performed in order to determine, whether the frameshift peptides arising from mutations in coding microsatellite regions according to the present invention are suited to stimulate a cellular immune response. The experiments were performed as follows:

15       Peptides displaying HLA-A2.1-binding motifs were selected by taking advantage of specific computer programs [(Parker, Bednarek, & Coligan 1994); [http://bimas.dcrf.nih.gov/molbio/hla\\_bind/](http://bimas.dcrf.nih.gov/molbio/hla_bind/) and (Rammensee et al. 1999); <http://134.2.96.221/scripts/MHCServer.dll/home.htm>]. Peptides were purchased from the peptide synthesis unit of the DKFZ. Stock solutions (10mg/ml in DMSO) were stored at -70°C and diluted to 1mg/ml in PBS before use. T2 cells were pulsed with 50µg/ml peptide and 5µg/ml β2-microglobulin (Sigma; Deisenhofen, Germany) overnight at 37°C. The expression of HLA-A2.1 was then analysed by flow cytometry using mAb BB7.2 followed by incubation with FITC-conjugated (ab')<sub>2</sub> goat anti-mouse Ig (Vonderheide et al. 1999).

20       Peripheral blood was obtained from a healthy HLA-A2.1+ donor and collected in heparinized tubes. PBMNC were isolated by Ficoll-density gradient centrifugation. Whole CD3+ T-cells were isolated from PBMNC by magnetic depletion of non-T-cells using the MACS Pan T-Cell Isolation Kit (Miltenyi; Bergisch Gladbach, Germany) according to manufacturer's instructions. Preparations contained at least 97% of CD3+ cells as assessed by immunophenotypic analysis.

30       CD40 Bs of a HLA-A2.1+ donor were incubated with peptide (10µg/ml) and human β2-microglobulin (3µg/ml; Sigma) in serum-free Iscov's DMEM medium for one hour at room temperature, washed twice to remove excess of peptide, were irradiated (30Gy) and added to purified CD3+ autologous T-cells (>97% CD3+) at a ratio of 4:1 (T:CD40 Bs) in Iscov's MEM containing 10% human AB-serum, supplements (1:100) and hIL-7 (10ng/ml, R&D). Cells were plated at a density of  $2 \times 10^6$  T-cells/well in 1ml of medium.

After three days in culture they were fed with <sup>44</sup>1ml complete medium. For restimulation of T-cells, this was repeated weekly. IL-2 was first given at day 21 (10IU/ml, R&D), also at day 24 and from day 28 on only hIL-2 was used instead of hIL7.

ELISpot assays were performed as described elsewhere (Meyer et al. 1998). Briefly,  
5 nitrocellulose-96-well plates (Multiscreen; Millipore, Bedford, USA) were covered with mouse anti-human IFN-g monoclonal antibody (Mabtech, Sweden) and blocked with serum containing medium. Varying numbers of effector cells were plated in triplicates with  $3,5 \times 10^4$  peptide-loaded T2 cells per well as targets. After incubation for 18h, plates were washed, incubated with biotinylated rabbit anti-human IFN-g second  
10 antibody, washed again, incubated with streptavidin coupled alkaline phosphatase, followed by a final wash. Spots were detected by incubation with NBT/BCIP (Sigma) for 45min, reaction was stopped with water, after drying spots were counted using the KS-ELISpot reader (Zeiss Kontron; Göttingen, Germany).

These procedures were performed for peptides derived from mutations in the coding  
15 regions of the following genes: TGF $\beta$ RII, OGT, U79260, CASP 5, MSH 3, HPDMPK, HT001, TAF1B, D070, MAC30X, FLT3L and SEC63. Peptides are listed in Figure 1. Results for ELISpot analysis is shown in Figure 2.

The analysis shows, that the used peptides are suited to raise an immune response. Peptides arising from frameshift mutations thus may be used to raise immune  
20 response for example in the course of vaccinations according to the present invention.

**Example 6: Cytotoxicity assay directed against cells displaying frameshift peptides.**

25 CTL bulk cultures and/or CTL clones obtained according to the method described in Example 1 were tested for their cytotoxicity as follows:

Due to the limited amount of cell material clones were in some experiments pooled for the determination of the cytotoxicity.

To obtain cells presenting frameshift peptides on the one hand different MSI+ cell lines  
30 endogenously expressing mutated mRNA of the respective frameshift peptides of TGF $\beta$ RII, OGT, U79260, CASP 5, MSH 3, HPDMPK, HT001, TAF1B, D070, MAC30X, FLT3L and SEC63 either expressed HLA-A2.1 endogenously or were stably

transfected with HLA-A2.1 on the other hand several MSI- cell lines expressing HLA-A2.1 were transfected with the mutated full-length cDNA of the respective frameshift peptides (of TGF $\beta$ RII, OGT, U79260, CASP 5, MSH 3, HPDMPK, HT001, TAF1B, D070, MAC30X, FLT3L and SEC63). After selection and expansion of the transfected cell lines for each respective frameshift peptide at least two stably transfected sub-cell lines were available. These cell lines were used in cytotoxicity assays, wherein negative controls were the respective untransfected MSI+ HLA-A2.1 negative and/or the MSI-, HLA-A2.1 positive cell lines.

It could be shown, that the transfected cell lines were lysed by the bulk cultures and/or pooled clones of CTLs. The reactivity was tested at different target cell to effector cell ratios. In average around 20%-30% of the target cells were lysed in the assays. The control cells were always lysed at a significantly lower percentage.

In Figures 3 and 4 results for the frameshift peptides FSP02 (TGF $\beta$ RII(-1)) and FSP06 (OGT(-1)) respectively are shown. These results shall be representative for the results related to the other frameshift peptides, which rendered similar rates of lysis.

The experiments show, that frameshift peptides may generate immune response. The frameshift peptides may furthermore be applied for the detection of the presence of cytotoxic T-cells directed against a particular frameshift peptide.

#### **Example 7: Screening for Antibodies directed against frameshift peptides in patient samples**

Serum of 25 patients with diagnosed colorectal carcinomas was tested for the presence of antibodies to a set of frameshift peptides arising from frameshift mutations in coding microsatellite regions of the following genes: TGF $\beta$ RII, U79260, CASP 5, HT001, PTHL3, MACS, TCF4, TAF1B, AC1, AIM2, SLC23A1, ABCF1, HSPC259, BAX, TCF6L1, FTL3L, OGT, ELAVL3, MAC30X, MAC30X, SLC4A3, PRKDC, UVRAG, MSH3 and SEC63. As a control the serum of 50 normal individuals was tested.

For the test synthetic peptides (20 to 40mers, partly overlapping) representing immunogenic portions of all relevant frameshift peptides (see figure 5) arising from the respective genes were spotted onto nylon membranes. The nylon membranes were

subsequently incubated for one hour in phosphate-buffered saline (PBS) with 5% milk powder for blocking unspecific membrane binding. After washing the membranes 3x with PBS, the membranes were incubated with the test and control sera. The sera were diluted 1:1.000 in PBS/0,5% milk powder and incubated overnight with gentle shaking. Subsequently the sera were removed, and membranes were washed three times in PBS before they were incubated with a polyclonal alkaline phosphatase conjugated goat anti-human IgG antibody for one hour. Thereafter, the membranes were washed repeatedly with PBS/0,05% TWEEN20 before staining reaction was developed using nitroblue tetrazolium chloride and bromochloro-indoyl-phosphate (SigmaAldrich) in Tris-buffered saline (TBS). Binding of human antibodies specific for individual frameshift polypeptides thus was made visible by color-deposit on the respective membrane.

- The results show, that in all samples of tumour patients antibodies directed against at least one peptide arising from frameshift mutations were present.
- This illustrates, that the method according to the present invention may be used for diagnosis of diseases associated with frameshift mutations in coding regions of genes.

We claim:

1. A nucleic acid sequence encoding a polypeptide selected from a group consisting of TAF1B, MACS, UVRAG, ELAVL3, TCF6L1, ABCF1, AIM2, CHD2, FLJ11053, KIAA1052, ACVR2 and HT001 having a frameshift mutation,
  - a. the insertion of one A in the A11 repeats of the genes TAF1B, MACS, HT001, FLJ11053, KIAA1052
  - b. the insertion of two A in the A11 repeats of the genes TAF1B, MACS, HT001, FLJ11053, KIAA1052
  - c. the deletion of one A in the A11 repeats of the genes TAF1B, MACS, HT001, FLJ11053, KIAA1052
  - d. the deletion of two A in the A11 repeats of the genes TAF1B, MACS, HT001, FLJ11053, KIAA1052
  - e. the insertion of one A in the A10 repeats of the genes CHD2, UVRAG, TCF6L1, ABCF1, AIM2
  - f. the insertion of two A in the A10 repeats of the genes CHD2, UVRAG, TCF6L1, ABCF1, AIM2
  - g. the deletion of one A in the A10 repeats of the genes CHD2, UVRAG, TCF6L1, ABCF1, AIM2
  - h. the deletion of two A in the A10 repeats of the genes CHD2, UVRAG, TCF6L1, ABCF1, AIM2
  - i. the insertion of one A in the A8 repeat of the gene ACVR2
  - j. the insertion of two A in the A8 repeat of the gene ACVR2
  - k. the deletion of one A in the A8 repeat of the gene ACVR2
  - l. the deletion of two A in the A8 repeat of the gene ACVR2
  - m. the insertion of one G in the G9 repeat of the gene ELAVL3
  - n. the insertion of two G in the G9 repeat of the gene ELAVL3
  - o. the deletion of one G in the G9 repeat of the gene ELAVL3
  - p. the deletion of two G in the G9 repeat of the gene ELAVL3
2. The nucleic acid according to claim 1 for use in detection of disorders associated with frameshift mutations in coding microsatellite regions and/or for use in preparation of pharmaceutical compositions for treatment of disorders associated with frameshift mutations in coding microsatellite regions.
3. A frameshift polypeptide selected from a group comprising:
  - a. a frameshift polypeptide derived from a frameshift mutation as described in claim 1 in a coding microsatellite region of a gene selected from the group consisting of CHD2, UVRAG, ELAVL3, TCF6L1, ABCF1, AIM2, TAF1B, MACS and HT001;
  - b. a polypeptide described by an amino acid sequence given in Figure 2;
  - c. a polypeptide encoded by a nucleic acid sequence according to claim 1;
  - d. a fragment or a portion of the polypeptides of a) to c) containing at least one amino acid not present in the wild-type protein



4. A frameshift polypeptide according to claim 3 for use in detection of disorders associated with frameshift mutations in coding microsatellite regions and/or for use in preparation of pharmaceutical compositions for treatment of disorders associated with frameshift mutations in coding microsatellite regions.
5. A method for treatment of disorders associated with frameshift mutations in coding microsatellites comprising administering one or more frameshift polypeptides according to claim 3 or nucleic acid sequences according to claim 1 in a pharmaceutical acceptable form to an individual.
6. The method of claim 5 additionally comprising administering one or more further frameshift polypeptides arising from frameshift mutations in coding microsatellite regions.
7. The methods according to the claims 5 or 6, wherein the disorder is a degenerative disorder
8. The methods according to the claims 5 or 6, wherein the disorder is a neurodegenerative disorder, vascular disease, cancer or precursory stages of cancer.
9. The methods according to any one of the claims 5-8, wherein the treatment is immuno-therapeutic treatment of disorders.
10. The methods according to any one of the claims 5-8, wherein the treatment is preventive vaccination against disorders.
11. A pharmaceutical composition for use in the methods according to any one of the claims 5-10 comprising a nucleic acid according to claim 1 and/or a polypeptide according to claim 3 in physiological acceptable form.
12. A method for detection of a disorder associated with frameshift mutations in coding microsatellite regions comprising detecting the presence or absence of one or more nucleic acids according to claim 1 and/or frameshift polypeptides according to claim 3 in a biological sample.
13. A method for detection of a disorder associated with frameshift mutations in coding microsatellite regions comprising detecting the presence or absence of antibodies directed against one or more frameshift polypeptides according to claim 3 in a biological sample.

14. A method for detection of a disorder associated with frameshift mutations in coding microsatellite regions comprising detecting the presence or absence of cells specifically directed against one or more frameshift polypeptides according to claim 3 in a biological sample.
- 5 15. The methods according to any one of the claims 12-14, wherein the disorder is a degenerative disorder
16. The methods according to any one of the claims 12-14, wherein the disorder is a neurodegenerative disorder, vascular disease, cancer or precursory stages of cancer.
- 10 17. A diagnostic or research kit for performing the methods according to any one of the claims 12-16 comprising one or more nucleic acids according to claim 1 and/or one or more polypeptides according to claim 3.
18. A method for treatment of disorders associated with peptides arising from frameshift mutations in coding microsatellite regions in individuals, comprising selecting a set of at least 5 different frameshift peptides according to the following requirements:
- 15
- a. at least 3 of the frameshift peptides occur with a frequency of more than 30% in tissues affected by said disorders;
  - b. the frameshift peptides comprise each at least one novel amino acid residue compared to the wild-type amino acid sequence; and
  - c. the frameshift peptides comprise members of at least 3 different biochemical pathways;
- 20
- and administering said frameshift peptides to an individual either as peptide or in form of a nucleic acid to be expressed in situ in a pharmaceutical acceptable form that enables induction of an immune response in the individual against the peptides.
- 25
19. The method of claim 18, wherein at least one coding microsatellite sequence is represented by peptides originating from at least two different reading frames of said single microsatellite region.

20. The method according to any one of the claims 18-19, wherein at least one frameshift peptide is derived from a frameshift mutation in the (A)<sub>10</sub> tract of the TGFβRII gene.
21. The method according to any one of the claims 18-20, wherein at least one frameshift peptide is derived from a frameshift mutation in the (G)<sub>8</sub> tract of the BAX gene.
22. The method according to any one of the claims 18-21, comprising at least five frameshift peptides selected from a group consisting of frameshift peptides derived from the (A)<sub>11</sub> tract of the MACS gene, the (A)<sub>10</sub> tract of the CASP5 gene, the (A)<sub>9</sub> tract of the TCF-4 gene, the (G)<sub>8</sub> tract of the IGF1R gene, the (T)<sub>10</sub> tract of the AC1 gene, the (A)<sub>9</sub> or (A)<sub>10</sub> tract of the SEC63 gene, the (A)<sub>11</sub> tract of the TAF1B gene, the (A)<sub>11</sub> tract of the PTHL3 gene, the (T)<sub>14</sub> tract of the U79260 gene, the (A)<sub>10</sub> tract of the AIM2 gene, the (A)<sub>10</sub> tract of the ABCF1 gene.
23. A method according to claim 18, wherein the set comprises frameshift peptides derived from mutations in the coding regions of the following genes: TGFβRII, U79260, CASP 5, HT001, PTHL3, MACS, TCF4, TAF1B, AC1 and SEC63
24. A method according to claim 18, wherein the set is selected from a group comprising
- |        |        |                   |
|--------|--------|-------------------|
| HT001  | U79260 | MACS              |
| HT001  | TAF1B  | MACS              |
| HT001  | TGFB2R | MACS              |
| HT001  | U79260 | TGFB2R            |
| HT001  | U79260 | TAF1B             |
| HT001  | TGFB2R | TAF1B             |
| HT001  | U79260 | TGFB2R MACS       |
| HT001  | U79260 | TGFB2R AC1        |
| HT001  | U79260 | TGFB2R TAF1B      |
| HT001  | TGFB2R | MACS CASP5        |
| HT001  | U79260 | MACS CASP5        |
| HT001  | U79260 | MACS AC1          |
| HT001  | TGFB2R | TAF1B CASP5       |
| HT001  | U79260 | MACS OGT          |
| U79260 | TGFB2R | AC1 CASP5         |
| HT001  | U79260 | TGFB2R MACS AC1   |
| HT001  | U79260 | TGFB2R TAF1B MACS |
| HT001  | U79260 | TGFB2R TAF1B AC1  |
| HT001  | U79260 | TGFB2R MACS AIM2  |
| HT001  | U79260 | TGFB2R TAF1B AIM2 |

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U79260	TGFB2R	TAF1B	AC1	CASP5
HT001	U79260	TGFB2R	AC1	CASP5
U79260	TGFB2R	MACS	AC1	CASP5
HT001	U79260	TAF1B	MACS	AC1
HT001	U79260	TAF1B	MACS	CASP5
HT001	U79260	MACS	AC1	OGT
HT001	U79260	MACS	MSH3	OGT
HT001	U79260	TGFB2R	MACS	OGT
HT001	TGFB2R	TAF1B	AC1	CASP5
HT001	U79260	TGFB2R	AC1	AIM2

25. The method according to claims 18 -24, wherein the disorder is cancer or its precursory stages.
26. The method according to claim 25, wherein the cancer is colon cancer.
27. The method according to claims 18-26, which is immuno-therapeutic treatment of disorders.
28. The method according to claims 18-27, which is preventive vaccination against disorders.
29. The method according to any one of the claims 18-28, wherein the nucleic acid administered to the individual is mRNA.
30. A pharmaceutical composition comprising a set of frameshift polypeptides as used in the methods of any one of the claims 18-24 for the use in immunotherapy according to claim 27 or 28 of disorders associated with frameshift mutations in coding microsatellite regions.
31. The pharmaceutical composition of claim 30, which is used for the treatment of cancer.
32. A method for the detection of a disorder associated with frameshift mutations in coding microsatellite regions in an individual comprising
- detection of the presence or absence and/or of the level of immunological entities specifically directed against one or more frameshift polypeptides originating from frameshift mutations in coding microsatellite regions in a biological sample, and
  - assessing diagnosis of the presence or absence of said disorder and/or prognosis of the disease course of said disorder from the presence or absence and/or the level of said immunological entities.

33. The method of claim 32, wherein the<sup>52</sup> immunological entity is an antibody or a fragment thereof.
34. The method of claim 32, wherein the immunological entity is a cell specifically directed against a frameshift polypeptide.
- 5 35. The method of any one of the claims 32-34, wherein the disorder is a neuro-degenerative disorder or cancer.
36. The method of claim 35, wherein the cancer is gastrointestinal cancer.
37. The method of any one of the claims 32-36, wherein immunological entities are specifically directed against at least one frameshift polypeptide derived from a frameshift mutation in the (A)<sub>10</sub> tract of the TGF $\beta$ RII gene.
- 10 38. The method of any one of the claims 32-37, wherein immunological entities are specifically directed against at least one frameshift polypeptide derived from a frameshift mutation in the (G)<sub>8</sub> tract of the BAX gene.
39. The method of any one of the claims 32-38, wherein immunological entities are specifically directed against at least one frameshift polypeptide selected from a group consisting of frameshift peptides derived from the (A)<sub>11</sub> tract of the MACS gene, the (A)<sub>10</sub> tract of the CASP5 gene, the (A)<sub>9</sub> tract of the TCF-4 gene, the (G)<sub>8</sub> tract of the IGFIIR gene, the (T)<sub>10</sub> tract of the AC1 gene, the (A)<sub>9</sub> or (A)<sub>10</sub> tract of the SEC63 gene, the (A)<sub>11</sub> tract of the TAF1B gene, the (A)<sub>11</sub> tract of the PTHL3 gene, the (T)<sub>14</sub> tract of the U79260 gene, the (A)<sub>10</sub> tract of the AIM2 gene, the (A)<sub>10</sub> tract of the ABCF1 gene.
- 15 40. The method of any one of the claim 32-39, wherein the detection is carried out in vitro.
41. The method of any one of the claim 32-39, wherein the detection is carried as an in situ immuno-cytochemical staining reaction.
- 20 42. A kit for performing the methods of any one of the claims 32-41, which is a diagnostic kit or a research kit.
- 25

Figure 1:

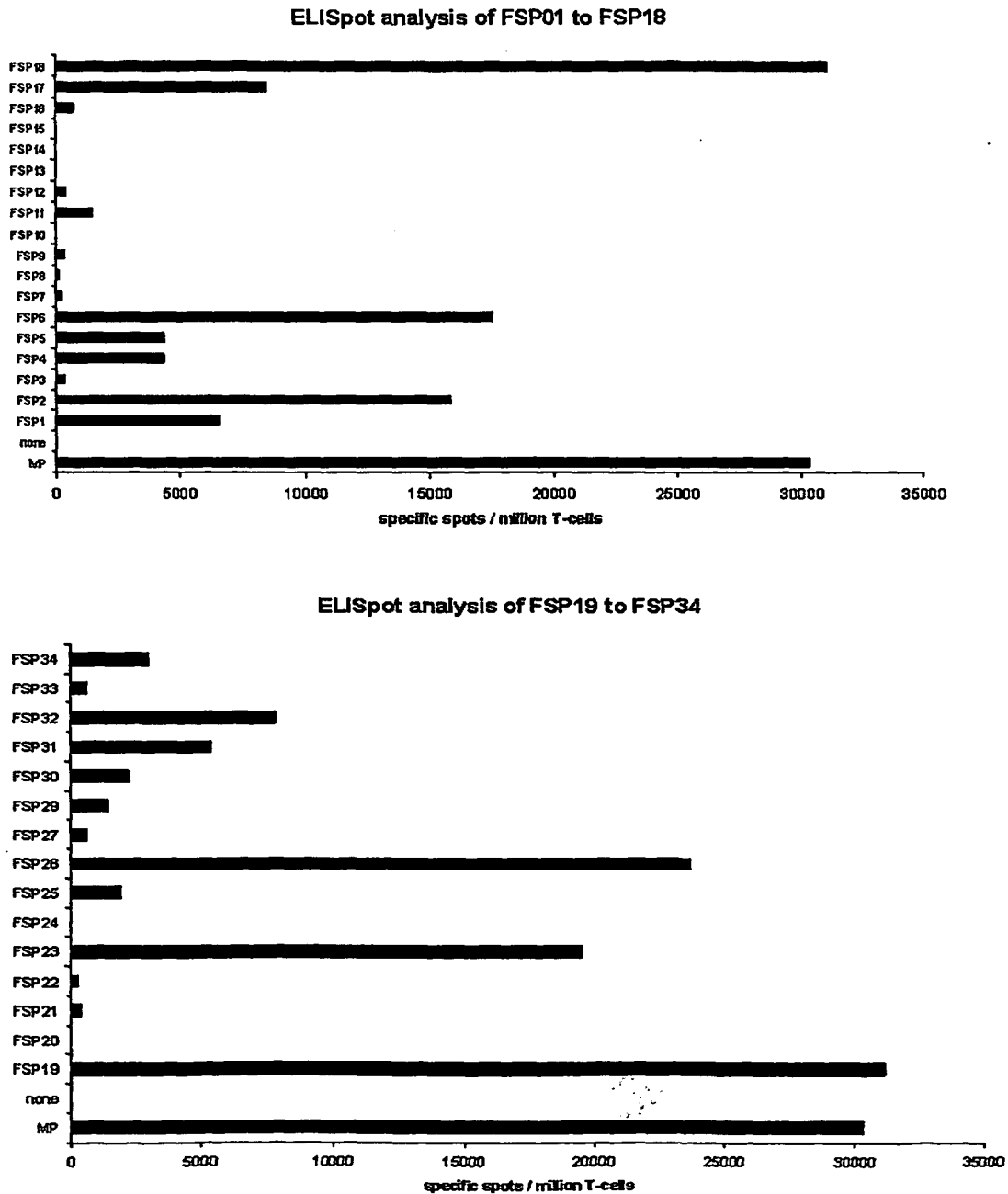


Figure 2

**HT001**  
 wt ORF  
 5 MQRPN AHRISQPIRQIYGLLLNASPHLDKTSWNALPPQPLAFSEVERINKNIRTSIIDAVELAKDHS DLSRLTELSLRRRQMLLLET LK  
 VKQTILEPIPTSLKLP IAVSCYWLQHTETKAKLHHLQSLLLTMLVGPLIAIINSPGKEELQEDGAKMLYAEFQRVKAQTRLGTRLDLDTA  
 HIFCQWQSC LQMGMYLNQLLSTPLPEPDLTRL YSGSLVHGLCQQLLASTSVESLLSICPEAKQLYEYLFNATRSYAPAEIFLPKGRS  
 NSKKKQKQKQNTSCSKNRGRTTAHTKCWYEGNNRFGLLMVENLEEHS EASNIE  
 (-1) ORF  
 10 MQRPN AHRISQPIRQIYGLLLNASPHLDKTSWNALPPQPLAFSEVERINKNIRTSIIDAVELAKDHS DLSRLTELSLRRRQMLLLET LK  
 VKQTILEPIPTSLKLP IAVSCYWLQHTETKAKLHHLQSLLLTMLVGPLIAIINSPGKEELQEDGAKMLYAEFQRVKAQTRLGTRLDLDTA  
 HIFCQWQSC LQMGMYLNQLLSTPLPEPDLTRL YSGSLVHGLCQQLLASTSVESLLSICPEAKQLYEYLFNATRSYAPAEIFLPKGRS  
 NSKKK~~GRRNRIPAVLRTEGEPLHTPSVGMRETTGLGC~~  
 (+1)/(-2) ORF  
 15 MQRPN AHRISQPIRQIYGLLLNASPHLDKTSWNALPPQPLAFSEVERINKNIRTSIIDAVELAKDHS DLSRLTELSLRRRQMLLLET LK  
 VKQTILEPIPTSLKLP IAVSCYWLQHTETKAKLHHLQSLLLTMLVGPLIAIINSPGKEELQEDGAKMLYAEFQRVKAQTRLGTRLDLDTA  
 HIFCQWQSC LQMGMYLNQLLSTPLPEPDLTRL YSGSLVHGLCQQLLASTSVESLLSICPEAKQLYEYLFNATRSYAPAEIFLPKGRS  
 NSKKK~~KIAETEYQLF~~

**U79260**  
 wt ORF  
 20 MGHPRAIQPSVFFSPYDVHFLLYPIRCPYLKIGRFHIKLGHLFLFSFLFFFETQSHSVTRLECSGTISAHCNLC LPGSSNSPASASR  
 VAGTAGTCRRAQLIFVFLAEMGFHHVGRDGLDNLVIHPPRSPKALGLQA  
 (-1) ORF  
 25 MGHPRAIQPSVFFSPYDVHFLLYPIRCPYLKIGRFHIKLGHLFLFSFLFFF~~LHSLTLSPGWSAVARSRLTATSASQVQVILLPOPPE~~  
~~WLGLQARAAAPS~~  
 (+1)/(-2) ORF  
 MGHPRAIQPSVFFSPYDVHFLLYPIRCPYLKIGRFHIKLGHLFLFSFLFFF(F)

**PTHL3**  
 (wt) ORF  
 30 MQRRLVQOWSVAVFLLSYAVPSCGRSVEGLSRRLKRAVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHAEIRATSEVSPNSKPSPN  
 TKNHVPRFGSDDEGRYLQTETNKVETYEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLD SGVTGSGLEGDHLSDTSTTSLEL  
 DSRTALLWGLKKKKENNRTHHMLQMLISLFSPLLLL  
 (-1) ORF  
 35 MQRRLVQOWSVAVFLLSYAVPSCGRSVEGLSRRLKRAVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHAEIRATSEVSPNSKPSPN  
 TKNHVPRFGSDDEGRYLQTETNKVETYEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLD SGVTGSGLEGDHLSDTSTTSLEL  
 DSRTALLWGLKKK~~RKITEEHIICN~~  
 (+1)/(-2) ORF  
 40 MQRRLVQOWSVAVFLLSYAVPSCGRSVEGLSRRLKRAVSEHQLLHDKGKSIQDLRRRFFLHHLIAEIHAEIRATSEVSPNSKPSPN  
 TKNHVPRFGSDDEGRYLQTETNKVETYEQPLKTPGKKKKGKPGKRKEQEKKKRRTRSAWLD SGVTGSGLEGDHLSDTSTTSLEL  
 DSRTALLWGLKKK(K)~~GKQKKNISYATNDLI~~

**TGFbRII**  
 (wt)  
 45 MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAV  
 WRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKPGGETFFMCSCSSDECNDNIIFSEYNTSNPDLLL VIFQVTGISLLPPL  
 GVAISVIIICYRVNRQQLSSTWETGKTRKLMFEFSEHCAIILED RSDISSTCANNININTELLPIELDTLVGKGRFAEYVYAKLKQN  
 TSEQFETAVKIFFYEEYASWKTEKDIFSDINLKHENILOFLTAERKTEL GKQYWLITAFHAKGNLQEYLTRHVISWEDLRKLGSSLA  
 50 RGI AHLHSDHTPCGRPKMPVHRDLNSSNVLKNDLTCCLCD FGLSLRLDPTLSVDDLANSGQVGTARYMAPEVLESRMNLENAES  
 FKQTDVYSMALVLWEMTSRCNAVGEVKDYEPFGSKVREHPCVESMKDNVLRDRGRPEIPSWFLNHQGIQMVCELTTECWDHDP  
 EARLTAQCAERFSELEHDLRLSGRSCSEKIPEDGSLNTTK  
 (-1) ORF  
 MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAV  
 WRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKK~~SLVRLSSCVPALMSAMTTSSSQKNITPAILTCC~~  
 (+1)/(-2) ORF  
 55 MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAV  
 WRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKK(K)~~QAW~~

**MACS**  
 (wt) ORF  
 60 MGAQFSKTAAKGEEAAERPGEEAVASSPSKANGQENGHVKNVGDASPA AAEESGAKEELQANGSAPAADKEEPAAGSGAASPS  
 SAEKGEPAAAAAPEAGASPVKEAPAEGEAAEPGSATAAEGEAASASSTSSPKAEDGATPSPSNETPKKKKKRFSFKSFKLSGF  
 SFKKNKKEAGEGEEAEAPAAEGGKDEAAGGAAAAAEEAGAAAGGEEAAGGDPQEA KPQEAAPAEKPPAS  
 DETKAAEESPKEVEEKAAEEAGASAAACEAPSAAGPAPPEQEAPAEPPAAAAASSACAAPSQEAQPECSPEAPPAAEAE  
 (-1) ORF  
 65 MGAQFSKTAAKGEEAAERPGEEAVASSPSKANGQENGHVKNVGDASPA AAEESGAKEELQANGSAPAADKEEPAAGSGAASPS  
 SAEKGEPAAAAAPEAGASPVKEAPAEGEAAEPGSATAAEGEAASASSTSSPKAEDGATPSPSNETPKKK~~RSAPPSRLSS~~  
 (+1)/(-2) ORF  
 70 MGAQFSKTAAKGEEAAERPGEEAVASSPSKANGQENGHVKNVGDASPA AAEESGAKEELQANGSAPAADKEEPAAGSGAASPS  
 SAEKGEPAAAAAPEAGASPVKEAPAEGEAAEPGSATAAEGEAASASSTSSPKAEDGATPSPSNETPKKK(K)~~EALFQEVFOAE~~  
~~RLLLQEEQEGGWRRR~~

## TCF-4

(wt)ORF

5 MPQLNGGGGDDLGADELISFKDEGEQEEKSSENSAERDLADVKSLLVNESETNQNSSDSEAERRPPPPRSEFRDKSRESLEE  
AAKRQDGGGLFKGPPYPGYPFIMIPDLTSPYLPNGSLSPARTYLQMKWPLLDVQAGSLQSRQALKDARSPSPAHIVSNKVPVQHP  
HHVHPLTPLITYSNEHFTPGNPPPHLPADVDPKTGIPRPPHPPDISPYPLSPGTVGQIPHPLGWLVPQQGQPVYPITGGFRHPYP  
TALTVNASVSRRFPPHMPVPHHTLHTTGIPHPAIVTPTVKQESSQSDVGSLSHSSKHQDSKKEEEKKKPHIKKPLNAFMYMKEMRAK  
VVAECTLKESAAINQILGRRWHALSREEQAKYYELARKERQLHMLQYPGWSARDNYGKKKKRKRDKQPGETNEHSECFNLNCLSL  
PPITDLAPKCKCRARFGLDQQNNWCGPCRRKKKSAFATYKVKAAASAHPLQMEAY

10 (-1)ORF

MPQLNGGGGDDLGADELISFKDEGEQEEKSSENSAERDLADVKSLLVNESETNQNSSDSEAERRPPPPRSEFRDKSRESLEE  
AAKRQDGGGLFKGPPYPGYPFIMIPDLTSPYLPNGSLSPARTYLQMKWPLLDVQAGSLQSRQALKDARSPSPAHIVSNKVPVQHP  
HHVHPLTPLITYSNEHFTPGNPPPHLPADVDPKTGIPRPPHPPDISPYPLSPGTVGQIPHPLGWLVPQQGQPVYPITGGFRHPYP  
TALTVNASVSRRFPPHMPVPHHTLHTTGIPHPAIVTPTVKQESSQSDVGSLSHSSKHQDSKKEEEKKKPHIKKPLNAFMYMKEMRAK  
VVAECTLKESAAINQILGRRWHALSREEQAKYYELARKERQLHMLQYPGWSARDNYGKKKKRKRDKQPGETNEHSECFNLNCLSL  
PPITDLAPKCKCRARFGLDQQNNWCGPCRRKKKSAFATYKVKAAASAHPLQMEAY

15 (+1)/(-2)ORF

MPQLNGGGGDDLGADELISFKDEGEQEEKSSENSAERDLADVKSLLVNESETNQNSSDSEAERRPPPPRSEFRDKSRESLEE  
AAKRQDGGGLFKGPPYPGYPFIMIPDLTSPYLPNGSLSPARTYLQMKWPLLDVQAGSLQSRQALKDARSPSPAHIVSNKVPVQHP  
HHVHPLTPLITYSNEHFTPGNPPPHLPADVDPKTGIPRPPHPPDISPYPLSPGTVGQIPHPLGWLVPQQGQPVYPITGGFRHPYP  
TALTVNASVSRRFPPHMPVPHHTLHTTGIPHPAIVTPTVKQESSQSDVGSLSHSSKHQDSKKEEEKKKPHIKKPLNAFMYMKEMRAK  
VVAECTLKESAAINQILGRRWHALSREEQAKYYELARKERQLHMLQYPGWSARDNYGKKKKRKRDKQPGETNEHSECFNLNCLSL  
PPITDLAPKCKCRARFGLDQQNNWCGPCRRKKK(VRS)HLTR

25 TAF1b

(wt)ORF

IPAFAGTVLQPFPEAALATRVTPAVEAPAAPRLDLEEESEEFKERCTQCAAVSWGLTDEGKYCTSCHNVTERYQEVNTNDLIPNT  
QIKALNRGLKJKKNTTEKGDWYVCEGFQYILYQQAELKNLGVGPELKNVHNFVKRYLQKSKQAYCKNPVYTTGRKPTVLEDN  
LSKSDWASEPELLSDVSCPPFLESQAESQSDIHTRKPPYVSKASQSETSVCSGSLDGVESQSRKEKGIVKMTMPQTALFCYLSLLW  
QREAITLSDLLRFVEEDHIPYNAFQHFPEQMKLYGRDRGIFGIESWPDYEDYKKTIEVGTFLDLPRFPDITEDCYLHPNLCMKYLM  
VNLPEMHSLTCHVVKMTGMGEVDFTFDPIAKMAKAVKYDVQAVAIIVVLKLLFLMDDSEFWSLSNLAEKHNEKNKDKPWFDF  
RKWYQIMKKAFADEKQKQWEEARAKYLWKSEKPLYYSFVDKPVAYKREMVNLQKQFSTLVDSTATAGKSPSSQFQNWTEEDT  
DRTCFHGHSLQGVLEKQGSLTKNSLYWLSTQKFCRW

35 (-1)ORF

IPAFAGTVLQPFPEAALATRVTPAVEAPAAPRLDLEEESEEFKERCTQCAAVSWGLTDEGKYCTSCHNVTERYQEVNTNDLIPNT  
QIKALNRGLKJKK(TL)KAGIGMCKVYSSIFINKQKP

(+1)/(-2)ORF

IPAFAGTVLQPFPEAALATRVTPAVEAPAAPRLDLEEESEEFKERCTQCAAVSWGLTDEGKYCTSCHNVTERYQEVNTNDLIPNT  
QIKALNRGLKJKK(KQ)Y

40 AC-1

(wt)ORF

MDTQKQIHKTHNSKNQFFTIFFLSVEFGKEGTRKNFYLLLSIGHYGRKSRRADLGTADTADKTEPECFAASWTFDPNPSVTVSGAH  
STAVHQ

45 (-1)ORF

MDTQKQIHKTHNSKNQFFTIFFSCQLNLGRKEHAKIFTFFQOLDTMDGNPGLTLELQTLQIKQSQNALPAGPLTQTPV

(+1)/(-2)ORF

MDTQKQIHKTHNSKNQFFTIFF(F)PVS

Sec63

(wt)ORF

50 MAGQQFQYDDSGNTFFYFLTSFVGLVIPATYYLWPRDQNAEQIRLKNIRKVVYGRCMWYRLRLKQPNIPTVKQVLLAGWALFLF  
LAYKVSKTDRYQYENPYEVLNLDPGATVAEIKQYRLLSLKYHPDKGGDEVFMFRIAKAYAALDEESRKNWEEFGNPDGPQAT  
SFGIALPAWIVDQKNSILVLLVYGLAFMVLVVGSWWYRSIRYSGDQILRTTQIYTYFVYKTRNMDMKRLIMVLGASEFDPQYNK  
DATSRPTDNILPQLIREIGSINLKQNEPPLTCPYSLKARVLLSLHARMKIPETLEEDQQFMLKKCPALLQEMVNVICQLVMARNREE  
REFRAPTLASLENCKMLSQMAVQGLQQFKSPLLQLPHIEEDNLRVSNHKKYKIKTIQDLVSLKESDRHTLLHLEDEKYEEVMAVL  
GSFFPVYTMIDKSQVLDDEDSNNITVGSVTVLVKLTRQTMAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQKSKGPKKTA  
55 KSKKKPLKPKPTPVLLPQSKQKQKQANGVVGNEAAVKEDEEEVSDKGSDEEEETNRDSQSEKDDGSDRDSREDEKQNK  
DDEAEWQELQSQIRKERALLETSKITHPVYSLYPPEEKQEWWWLYADRKEQTLISMPYHVCTLDTEEVEVKFPAPGPKPNYQ  
YTVFLRSDSYMGLDQIKPLKLEVHEAKVPENHPQWDTAIEGDEQEDQSEGFEDSFEEDDDDD

(-1) 9er A-Repeat

60 MAGQQFQYDDSGNTFFYFLTSFVGLVIPATYYLWPRDQNAEQIRLKNIRKVVYGRCMWYRLRLKQPNIPTVKQVLLAGWALFLF  
LAYKVSKTDRYQYENPYEVLNLDPGATVAEIKQYRLLSLKYHPDKGGDEVFMFRIAKAYAALDEESRKNWEEFGNPDGPQAT  
SFGIALPAWIVDQKNSILVLLVYGLAFMVLVVGSWWYRSIRYSGDQILRTTQIYTYFVYKTRNMDMKRLIMVLGASEFDPQYNK  
DATSRPTDNILPQLIREIGSINLKQNEPPLTCPYSLKARVLLSLHARMKIPETLEEDQQFMLKKCPALLQEMVNVICQLVMARNREE  
REFRAPTLASLENCKMLSQMAVQGLQQFKSPLLQLPHIEEDNLRVSNHKKYKIKTIQDLVSLKESDRHTLLHLEDEKYEEVMAVL  
GSFFPVYTMIDKSQVLDDEDSNNITVGSVTVLVKLTRQTMAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQKSKGPKKTA  
65 KSKKANL

(+1)/(-2) 9er A-Repeat

70 MAGQQFQYDDSGNTFFYFLTSFVGLVIPATYYLWPRDQNAEQIRLKNIRKVVYGRCMWYRLRLKQPNIPTVKQVLLAGWALFLF  
LAYKVSKTDRYQYENPYEVLNLDPGATVAEIKQYRLLSLKYHPDKGGDEVFMFRIAKAYAALDEESRKNWEEFGNPDGPQAT  
SFGIALPAWIVDQKNSILVLLVYGLAFMVLVVGSWWYRSIRYSGDQILRTTQIYTYFVYKTRNMDMKRLIMVLGASEFDPQYNK  
DATSRPTDNILPQLIREIGSINLKQNEPPLTCPYSLKARVLLSLHARMKIPETLEEDQQFMLKKCPALLQEMVNVICQLVMARNREE  
REFRAPTLASLENCKMLSQMAVQGLQQFKSPLLQLPHIEEDNLRVSNHKKYKIKTIQDLVSLKESDRHTLLHLEDEKYEEVMAVL  
GSFFPVYTMIDKSQVLDDEDSNNITVGSVTVLVKLTRQTMAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQK  
KSGKPKTKAKSKK(K)ETFKKTYTCAITTVKATETKAGKWSRWE

(-1) 10er A-Repeat



MAGQQFQYDDSGNTFFYFLTSFVGLVIPATYYLWPRDQNAEQIRLKNIRKVVYGRCMWYRLRLKPQPNIIPTVKKIVLLAGWALFLF  
LAKVSKTDREYQEYNPYEVLNLDPGATVAEIKKQYRLLSLKYHPDKGGDEVFMFRIAKAYAALTDDESRKNWEEFGNPDGPQAT  
SFGIALPAWIVDQKNSILVLLVYGLAFMVLVVGSGWWYRSIRYSGDQILIRTTQIYTYFYVYKTRNMDMKRLIMVLAGASEFDPQYNK  
5 DATSRPTDNILIPQLIREIGSINLKKNEPPLTCPYSLKARVLLSHLARMKIPETLEEDQQFMLKKOPALLQEMVNVICQLVIMARNREE  
REFRAPTLASLENCMKLSQMAVQGLQQFKSPLLQLPHIEEDNLRVSNHKKYKIKTIQDLVSLKESDRHTLLHLEDEKYEVEVMAVL  
GSFPYVTMDIKSQVLDDEDSNNITVGSVTVLVKLTRQTMAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQQ  
KSKGPKKTKAKSKKKPLKKNHLCCYYHSQSNRKNKSROMESLGMKLQ

(+1)/(-2) 10er A-Repeat

MAGQQFQYDDSGNTFFYFLTSFVGLVIPATYYLWPRDQNAEQIRLKNIRKVVYGRCMWYRLRLKPQPNIIPTVKKIVLLAGWALFLF  
LAKVSKTDREYQEYNPYEVLNLDPGATVAEIKKQYRLLSLKYHPDKGGDEVFMFRIAKAYAALTDDESRKNWEEFGNPDGPQAT  
10 SFGIALPAWIVDQKNSILVLLVYGLAFMVLVVGSGWWYRSIRYSGDQILIRTTQIYTYFYVYKTRNMDMKRLIMVLAGASEFDPQYNK  
DATSRPTDNILIPQLIREIGSINLKKNEPPLTCPYSLKARVLLSHLARMKIPETLEEDQQFMLKKOPALLQEMVNVICQLVIMARNREE  
REFRAPTLASLENCMKLSQMAVQGLQQFKSPLLQLPHIEEDNLRVSNHKKYKIKTIQDLVSLKESDRHTLLHLEDEKYEVEVMAVL  
15 GSFPYVTMDIKSQVLDDEDSNNITVGSVTVLVKLTRQTMAEVFEKEQSICAAEEQPAEDGQGETNKNRTKGGWQQ  
KSKGPKKTKAKSKKKPLKK(K)TYTCAITVKATETKAGKWSRWE

### Caspase 5

(wt)ORF

MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKDNHKKTKVMLEYLKGKDVHLGVFNLYLAKHDVLTKEEEKKKY  
YDAKIEDKALVDSLRKNRVAHQMFQTLLNMDQKTSVKPLQIEAGPPESAESTNKLCPREEFLRLCKKNHDEIPIKKREDRR  
20 RLALICNTKFDHLPARNGAHYDIVGMKRLQGLGYTVDEKNTLARDMESVLRFAARPEHKSSDSTFLVLSHGILEGICGTAHKK  
KPDVLLYDTIIPQILREIGSINLKKNEPPLTCPYSLKARVLLSHLARMKIPETLEEDQQFMLKKOPALLQEMVNVICQLVIMARNREE  
WRDRTGRSIFTELTCFQKYSKCCCHLMEIFRKVQKSFEVPOAKAQMPPTIERATLTRDFYLPFGN

(-1)ORF

MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKDNHKKQLRCWNTWAKMFMFLIHWQNTMF

(+1)/(-2)ORF

MFKGILQSGLDNFVINHMLKNNVAGQTSIQTLVPNTDQKSTSVKKDNHKK(K)NS

### AIM2

(wt)ORF

MESKYKEILLTGLDNITDEELDRFKFFLSDEFNIATGKLHTANRIQVATLMIQNAGAVSAVMKTIKIRIFOKLNYMILLAKRLQEEKEKVDK  
QYKSVTKPKPLSQAEMSPAASAAIRNDVAKQRAAPKVSHPVKPEQKQMVAAQESIREGFQKRCPLVMVLKAKKPFETFQEGKQE  
30 MFHATVATEKEFFVVKVFNLTLLKDKFIPKRIIARIYRHSGFLEVNSASRVLDAESDQKVNVPNIIRKAGETPKINTLQTQPLGTTVNG  
LFVQKVTEKKNILFDLSDNTGKMEVLGVRNEDTMCKEKGDKVRLTFFTLKNGEKLQLTSGVHSTIKVIKAKKK

(-1)ORF

MESKYKEILLTGLDNITDEELDRFKFFLSDEFNIATGKLHTANRIQVATLMIQNAGAVSAVMKTIKIRIFOKLNYMILLAKRLQEEKEKVDK  
35 QYKSVTKPKPLSQAEMSPAASAAIRNDVAKQRAAPKVSHPVKPEQKQMVAAQESIREGFQKRCPLVMVLKAKKPFETFQEGKQE  
MFHATVATEKEFFVVKVFNLTLLKDKFIPKRIIARIYRHSGFLEVNSASRVLDAESDQKVNVPNIIRKAGETPKINTLQTQPLGTTVNG  
LFVQKVTEKKNILFDLSDNTGKMEVLGVRNEDTMCKEKGDKVRLTFFTLKNGEKLQLTSGVHSTIKVIKAKKKHREYKRTNSSQ

LV

(+1)/(-2)ORF

MESKYKEILLTGLDNITDEELDRFKFFLSDEFNIATGKLHTANRIQVATLMIQNAGAVSAVMKTIKIRIFOKLNYMILLAKRLQEEKEKVDK  
40 QYKSVTKPKPLSQAEMSPAASAAIRNDVAKQRAAPKVSHPVKPEQKQMVAAQESIREGFQKRCPLVMVLKAKKPFETFQEGKQE  
MFHATVATEKEFFVVKVFNLTLLKDKFIPKRIIARIYRHSGFLEVNSASRVLDAESDQKVNVPNIIRKAGETPKINTLQTQPLGTTVNG  
LFVQKVTEKKNILFDLSDNTGKMEVLGVRNEDTMCKEKGDKVRLTFFTLKNGEKLQLTSGVHSTIKVIKAKKK(K)NIKE

### SLC23A1

(wt)ORF

MMGIGKNTTSKSMAGSSTEGKYEDEAKHPAFFTLPVVINGGATSSGEQDNEDTELMAIYTTENGIAEKSSLAETLDSTGSLDPQRS  
DMYITIEDVPPWYLCIFLGLQHYLTCSFGTIAPFLADAMCVGYDQWATSQIGTIFFCVGITTLLQTTFGCRPLPLFQTSFAFLAPAR  
45 AILSLDKWKCNNTDVSANGTAELLHTEHIWYPRIREIQGAIMSSLIEVVIGLLGLPGALLKYIGPLTTTPTVALIGLSGFOAAGERAGKH  
WGIAMLTIFLVLLFSQYARNVKFPLPIYKSKKGWTAAYKLQLFKMFPIILAILVSWLLCFITVTDVFPDPSTKYGFYARTDARQGVLLVA  
50 PWFVKVPYFQWGLPTVSAAGVIGMLSAVVASIESIGDYACARLSCAPPPPSTQ  
PWFKVPYFQWGLPTVSAAGVIGMLSAVVASIESIGDYACARLSCAPPPP  
GITKVGSRVQCGAALMLALGMIGKFSALFASLPDVLGALFCTFLGMITAVGLSNLOFIDLNSRNLFLVGFISIFFGLVPLSYLRQNL  
PLVTGITDQVNLVLLTTAMFVGCGVAFILDNTIPGTPEERGIRKWKKGVGKGNKSLDGMESYNLPFGMNIKKYRCFSYLPISPTFV  
GYTWKGLRKSDNSRSDSDSQATG

(-1)ORF

MMGIGKNTTSKSMAGSSTEGKYEDEAKHPAFFTLPVVINGGATSSGEQDNEDTELMAIYTTENGIAEKSSLAETLDSTGSLDPQRS  
55 DMYITIEDVPPWYLCIFLGLQHYLTCSFGTIAPFLADAMCVGYDQWATSQIGTIFFCVGITTLLQTTFGCRPLPLFQTSFAFLAPAR  
AILSLDKWKCNNTDVSANGTAELLHTEHIWYPRIREIQGAIMSSLIEVVIGLLGLPGALLKYIGPLTTTPTVALIGLSGFOAAGERAGKH  
WGIAMLTIFLVLLFSQYARNVKFPLPIYKSKKGWTAAYKLQLFKMFPIILAILVSWLLCFITVTDVFPDPSTKYGFYARTDARQGVLLVA  
60 PWFVKVPYFQWGLPTVSAAGVIGMLSAVVASIESIGDYACARLSCAPPPPSTQ

(+1)/(-2)ORF

MMGIGKNTTSKSMAGSSTEGKYEDEAKHPAFFTLPVVINGGATSSGEQDNEDTELMAIYTTENGIAEKSSLAETLDSTGSLDPQRS  
65 DMYITIEDVPPWYLCIFLGLQHYLTCSFGTIAPFLADAMCVGYDQWATSQIGTIFFCVGITTLLQTTFGCRPLPLFQTSFAFLAPAR  
AILSLDKWKCNNTDVSANGTAELLHTEHIWYPRIREIQGAIMSSLIEVVIGLLGLPGALLKYIGPLTTTPTVALIGLSGFOAAGERAGKH  
WGIAMLTIFLVLLFSQYARNVKFPLPIYKSKKGWTAAYKLQLFKMFPIILAILVSWLLCFITVTDVFPDPSTKYGFYARTDARQGVLLVA  
PWFVKVPYFQWGLPTVSAAGVIGMLSAVVASIESIGDYACARLSCAPPPP

(P)HPRNKQGNFRGRPLLS

### ABC1

(wt)ORF

MPKAPKQPPPEWIGDGESTSPSKVVKKGKKDKKIKKTFEELAVEDKQAGEEEKVLKEKEQQQQQQQKQKRDTRKGRR  
70 KQDVEDDGEKELMERLKKLSVPTSDEEDVPAKPRGGKKTGKGNVFAALIQDQSEEEEEEEKHPPKPAKPEKNRIINKAVSEEQ  
QPAKKGKKGKEEKSQKAKPQNKFAALDNEEDKEEIIKEKPPKQKQKAKKAEQMEYERQVASKAANAENDFSVSOAEMS  
SRQAMLENASDIKLEKFSISAHGKELFVNADLYVAGRRYGLVGPNGKGTLLKHIANRALSIPPNDIVLCEQEVVADETPAVQAVL  
RADTKRLKLEEFERLLQYQLEQGDQDAAERLEKVEELRATGAFAAAEAKARRILAGLGFDEMQNRPTQKFSGGWRMRVSLARAL  
75 FMEPTLLMLDEPTNHLDLNAVWLNLYLQGWRTLLVSHDQGLDDVCTDIIHLDAQRLHYRGNMTRFKMYQQKQKELLKQYE  
KQEKLLKEIKAGGKSTKQAEKQTKALTRKQKQCRKKNQDEESQEAPELLKRPKEYTVRFTFPDPPLSPPVGLHGVTFGYQGO

[illegible]

## ELAVL3

(wt)ORF

MESQVGGGPAGRPAPRPLLTNGATDDSKTNLVNYPQNMTQDEFKSLFSGSIGDIESCKLVRDKITGQSLGYGFVNYSNDPNDADK  
 AINTNLGLKLTQTKIKVSYARPPSSASIRDANLYVSGLPKTMQKEMEQLFSQYGRITSRILVDQVTGVSRRGVGFIRFDKRIEAEAEIKG  
 LNGQKPLGAREPITVKFANNPSOKTGOALLTHLYQSSARRYAGPLHHQTRFRDLNLLNMAYAVKRFSPADGMSGLAGVLSGG  
 AAGGWCFVYNLSPEPDQSVLWQLFGPFGAVTNVVKIRDFTTNKCKGFGFMTMTNYDEAAMAIASLNGYRLGQVRVLQVSFKTSKQ  
 HKA

(-1)ORF

MESQVGGGARPAGLPNGHSLVOMEPLTTARPTSSSTTCPRIT

(+1)/(-2)ORF

MESQVGG(G)PGRPACPTATPWYKWSH

MAC30X

(wt)ORF

LFSHQVRVQAQPTDYGGSFTRRCVEWLLGLYFLSHIPITLMDLQAVVPRELYPVEFRNLLKWYAKEFKDPLLQEPAPWFKSFLFCEL  
 VFQLPFFPIATYAFKLGSCWKWIRTPAIYSVHTMTTLILSTLFEDFSKASGFGQRPETLHERLTLVSVYAPYLLIPFILLFMLRSPYY  
 KYEEKRKKK

(-1)ORF

LFSHQVRVQAQPTDYGGSFTRRCVEWLLGLYFLSHIPITLMDLQAVVPRELYPVEFRNLLKWYAKEFKDPLLQEPAPWFKSFLFCEL  
 VFQLPFFPIATYAFKLGSCWKWIRTPAIYSVHTMTTLILSTLFEDFSKASGFGQRPETLHERLTLVSVYAPYLLIPFILLFMLRSPYY  
 KYEEKRKKKNEGNHWPVEMFTGWLVLGVYQEHCEPTSSAAFTLAAMHKSMMVSGTMSNPILLPFFFFF

(+1)/(-2)ORF

LFSHQVRVQAQPTDYGGSFTRRCVEWLLGLYFLSHIPITLMDLQAVVPRELYPVEFRNLLKWYAKEFKDPLLQEPAPWFKSFLFCEL  
 VFQLPFFPIATYAFKLGSCWKWIRTPAIYSVHTMTTLILSTLFEDFSKASGFGQRPETLHERLTLVSVYAPYLLIPFILLFMLRSPYY  
 KYEEKRKKKMKETTTGPG

SLC4A3

(wt)ORF

MANGVIPPGGASPLPQVRVPLEEPPLSPDVEEEDDGLKTLAVSRFGDLISKPPAWDPEKPSRSYSERDFEFHRTSHHTHPLS  
 ARLPPPHKLRLPPTSARHTRRKKEKTSAPPSGTPPIQEEGGAGVDEEEEEEEEEGESEAEPEPPPGSGTPQKAKFSIGSDE  
 DDSPGLPGRAAVTKPLPSVGPHTDKSPQHSSSSPRARASRLAGEKSRFPWSPASDYDLRERLCPGSALGNPGGPEQVPTDEA  
 EAQMLGSADLDDMKSHRLEDNPGVRRHLVKKPSRTQGGRGSPSGLAPILRRKKKKKLDRRPHEVFVELNELMLDRSQEPHWRE  
 TARWIKFEEDVEEETERWGPKHVASLSFRSLELRRTIAHGAALLDEQTTLPGLAHLVETMIVSDQIRPEDRASVLRLLKHSHPN  
 DDKDSGFFPRNPSSSMNSVLGNHPTPSHGPDGAVPTMADDLGEAPLWPHDPDAKEKPLHMPGGDGHGKSLKLEKIPEDA  
 EATVVLVGCVPFLEQPAAPFRLNEAVLLESVLEVPVVRFLVFLVMLGPSHTSTDYHELGRSIATLMSDKLFHEAAYQADDRODLSAI  
 SEFLDGSVIPPSEVEGRDRLRSVAAFORELLRKRREEREQTKVEMTTRGGYTAPGKELSELGGSEATPEDDPLRTGSVFGGLVR  
 DVRRRYPHYPSDLRDLHSCQVAAVLFYFAALSPAITFGGLGEKTEGLMGVSELVSTAVLGVLSLLGAQPLLVVGFSGPLVFE  
 EAFKFCRAQDLEYLTGRVWVGLWLVFVLAALVAAEGSFLVRYISPTQEIFAFLISLIFYETFYKLYKVFEHPPLPFYPPEGALEGS  
 LAAGLEPNGSALPPTGPPSPRNQNTALLSLJLMLGTFFIAFLRFRNSRFLGGKARRIIGDFGIPISILVMVLVDYSITDTYTQKLT  
 PTGLSVTSPDKRSWFIPPLGSAFPFPWMMVAAVPAVLLVLIJFMETQITALVSVQKARRLLKSGFHLDLLJLSGLGGLCGFLGLPW  
 LTAATVRSVTHVNAITVMRTAIAPGDKPQIQEVREQRVTGVLIASLVGLSIVMGAVLRRIPAVLFGIFLYMGVTSLSGQILSQRLLLIL  
 MPAKHHPPEQPYVTKVKTWRMHLFTCIQLGCIALLWVVKSTAASLAPFLLLTVPLRHCLLPRLFQDRELQALDSEDAEPNFDDEGQ  
 DEYNELHMPV

(-1)ORF

MANGVIPPGGASPLPQVRVPLEEPPLSPDVEEEDDGLKTLAVSRFGDLISKPPAWDPEKPSRSYSERDFEFHRTSHHTHPLS  
 ARLPPPHKLRLPPTSARHTRRKKEKTSAPPSGTPPIQEEGGAGVDEEEEEEEEEGESEAEPEPPPGPHRRQSSPLEVT  
 RMTVQASLGGLLSPSPCPRAWHTL TRAPSTPAAPPAGPGPPDSLGRKAGPGAHRPVMTCGSDCAGAVPWATOVVQSSRCQOM  
 RRRPRCWVLTQWTT

(+1)/(-2)ORF

MANGVIPPGGASPLPQVRVPLEEPPLSPDVEEEDDGLKTLAVSRFGDLISKPPAWDPEKPSRSYSERDFEFHRTSHHTHPLS  
 ARLPPPHKLRLPPTSARHTRRKKEKTSAPPSGTPPIQEEGGAGVDEEEEEEEEEGESEAEPEPP(P)LRDPTGKVLHWK

PRKDC

(wt)ORF

MAGSGAGVRCSLRLQETLSAADRCGAALAGHQLIRGLGQECVLSSSPAVLALQTSLVFSRDFGLLVFVRKSLNSIEFRECREEILK  
 FLCIFLEKMGQKIAPYSVEIKNTCTSVYTKDRAAKCKIPALDLLIKLTFRSSRLMDEFKIGELFSKFYGEALAKKKIPDVTLEKVVYELL  
 GLLGEVHPSEMINNAENLFRAGLGTQMTSAVREPPLVLAGCLKGLSLLCNFTKSEEDPQTSREIFNFVLKAIRPQIDLKRYA  
 VPSAGLRFLFALHASQFSTCLLDNYVSLFEVLLKWCATHTNVELKKAALSALLESFLKQVSNMVAKNAEMHKNLQYFMEQFYGIIRNVD  
 SNINKELSIARGYGLFAGPCKVINAKDVDFMYVELIQRCKQMFQTQDTGDDRVYQMPFLQSVASVLLYDTPVEVYTPVLEHLVV  
 MQIDSFPQYSPKMLVCCRAIVKFLAALAGPVLNRCISTVHQLIRICSKPVVLPKGPESSESDHRASGEVVRTGKWKVPTYKYDY  
 VDLFRHLLSSDQMMDSILADEAFFSVNSSSESLLNLLYDEFVKSVLKVEKLDLTLEIQTVGEQENGDEAPGVWMIPTSDPAANLHPA  
 KPKDFAFINLVEFCREILPEKQAEFFEPWVYSFSEYELJQSTRPLISGFYKLLSITVRNKKIKYFEGS

(-1)ORF

MAGSGAGVRCSLRLQETLSAADRCGAALAGHQLIRGLGQECVLSSSPAVLALQTSLVFSRDFGLLVFVRKSLNSIEFRECREEILK  
 FLCIFLEKMGQKIAPYSVEIKNTCTSVYTKDRAAKCKIPALDLLIKLTFRSSRLMDEFKIGELFSKFYGEALAKKKYQIQF

(+1)/(-2)ORF

MAGSGAGVRCSLRLQETLSAADRCGAALAGHQLIRGLGQECVLSSSPAVLALQTSLVFSRDFGLLVFVRKSLNSIEFRECREEILK  
 FLCIFLEKMGQKIAPYSVEIKNTCTSVYTKDRAAKCKIPALDLLIKLTFRSSRLMDEFKIGELFSKFYGEALAKKK(NTRYSFKSI

UVRAG

(wt)ORF

MSASASVGGPVPPPPGPAALPPGSAARALHVELPSQQRRLRLHRLNIAARNIVNRNGHQLLDYFTLHLCSTEKIKYEFYRSEVIK  
 NSLNPTWRSRDLDFGIMPDRDTSVSCFVVKWGGKENIYQLLJEWKVCGLDGLKYLQGOIHARNQNEIIFGLNDGYGAPFEHKGYGNA  
 QKTILLQVDQNCVRNSYDVFSLLRLHRAQCAIKQTQVTQKIGKEIEEKLRLTSTSNELKKKSECLQLKILVLONELERQKALGREVA  
 LLHKQALQDKGSAFSAEHLKLLQKESNELRKECTAKRELFLKTNALQTLTRCQLLSSELYIPIDLNEHKDYFCGVKLPNSDF  
 QAKDDGSIIVALGYTAHLVSMISFLLQVPLRYPIIHKGSRTIKDNINDKLTEKEREFFLYPKGGKELQFDYGVYLLNKINIAQLRYQHG  
 LGTPDLRQTLPLNKNFMHGLMVRCDRIHTSSAIPVPRKQSSIFGGADVGFSGGIPSPDKGHRKRASSENERLQYKTPPPSYNSAL  
 AQPVTTPVSMGETERIKTSLSSSLDLSLDFSKENKKKGEDLVGSLNGGHANVHPSQEQQEALSGHRATVNGTLLPSEQAGSASVQ  
 LPGEFHPVSEAELOCTVEQAEELGLEAQVSPQVIS

## (-1)ORF

MSASASVGGPVPQPPPGPAAALPPGSAARALHVELPSQQRRLRHLRNLIAARNIVNRNGHQLLDITYFTLHLCSTEKIYKEFYRSEVIK  
NSLNPTWRSDFGIMPDRDLSVSCFVVKWGGKENIYQLLIEWKVCGLDGLKYLGGQIHARNQNEIFGLNDGGYGAFFEHKGYSNA  
QKTILLQVDQNCVRNSYDVFSLRLHRAQCAIKQTQVTVQKIGKEIEEKLRLTSTSNELKKK(VNACS)

## (+1)(-2)ORF

MSASASVGGPVPQPPPGPAAALPPGSAARALHVELPSQQRRLRHLRNLIAARNIVNRNGHQLLDITYFTLHLCSTEKIYKEFYRSEVIK  
NSLNPTWRSDFGIMPDRDLSVSCFVVKWGGKENIYQLLIEWKVCGLDGLKYLGGQIHARNQNEIFGLNDGGYGAFFEHKGYSNA  
QKTILLQVDQNCVRNSYDVFSLRLHRAQCAIKQTQVTVQKIGKEIEEKLRLTSTSNELKKK(K)

## MSH3

## (wt)ORF

MSRRKPASGGLAASSAPARQAVLSRFFQSTGSLKSTSSSTGAADQVDPGAAAAAAAAAAPPAPPAPAFPPLPPHVATEIDR  
RKKRPLENDGPVKKVKKVQKQEGGSDLGMSGNSEPKCLRTNRVSKSLEKJKEFCDSALPQSRVQTESLQERFAVLPKCTDFD  
DISLLHAKNAVSSSESKRQINQKDTTLFDLSQFGSSNTSHENLQKTASKSANKRSKSIYTPLELQYIEMKQHQHDAVLCVECGYKYR  
FFGEDAEIAARELNICYCHLDHNFMTASIPHTRLFVHVRLVAKGYKGVVVKQTETAAKKAIGDNRSSLFSRKLALYTKSTLIGEDVNP  
LIKLDADVNDDEIMTDTSTSYLLCISENKENVRDKKKNIFIGIVGVPATGEVVFDSFQDSASRSELETRMSSLQPVLLPSALSEQ  
TEALHFRATSVSVQDDRIVRVERMDNIYFEYSHAFQAVTEFYAKOTVDIKGSQIISGIVNLEKPVICSLAAIKYLKEFNLEKMLSKPENFK  
QLSSKMEFMTINGTTLRNLEILONQDMDKTKGSLVWLDHTKTSFGRRKLKQWVTOPLKLRINARLDAVSEVLHSESSVFGQIEN  
HLRKLDPIDGRGLCSIYHKKSTOEFLVKTLYHLKSEFQAIIPAVNSHISQSDLLRTVILEIPELLSPVEHYLKILNEQAQKVGDKTELKFD  
LSDFPKIKRDEIQGVDEIRMHLEIRKILKNPSAQYVTVSGQEFMIEIKNSAVSCIPTDWWKVGSTKAVSRFHSPPFIVENYRHLNQL  
REQLVLDCSAEWLDFLEKFESEYHSLCKAVHHLATVDCIFSLAKVAKQGDYCRPTVQEEERKIVIKNGRHPVIDVLLGEQDQVYPNNT  
DLSEDSERVMIITGPNMGGKSSYKQVALTIMAQISYVPAEEATIGIVDGIPTRMGAADNIYKGRSTFMEELTDTAEIIRKATSSQLVL  
DELGRGTSTHDGIAIAYATLEYFIRDVKSLLTFVTHYPPVCELEKNYSHQVGNHYMGFLVSEDESKLDPGTAEQVDFVTFLYQITRG  
IAARSYGLNVAKLADVPGEILKKAHKSKELEGLINTKRKLKYFAKLWTMHAQDLQKWTEEFNMEETQTSLLH

## (-1)ORF

MSRRKPASGGLAASSAPARQAVLSRFFQSTGSLKSTSSSTGAADQVDPGAAAAAAAAAAPPAPPAPAFPPLPPHVATEIDR  
RKKRPLENDGPVKKVKKVQKQEGGSDLGMSGNSEPKCLRTNRVSKSLEKJKEFCDSALPQSRVQTESLQERFAVLPKCTDFD  
DISLLHAKNAVSSSESKRQINQKDTTLFDLSQFGSSNTSHENLQKTASKSANKRSKSIYTPLELQYIEMKQHQHDAVLCVECGYKYR  
FFGEDAEIAARELNICYCHLDHNFMTASIPHTRLFVHVRLVAKGYKGVVVKQTETAAKKAIGDNRSSLFSRKLALYTKSTLIGEDVNP  
LIKLDADVNDDEIMTDTSTSYLLCISENKENVRDKK(RATFLLALWECSLPQARLCLIVSRILLVOS)

## (+1)(-2)ORF

MSRRKPASGGLAASSAPARQAVLSRFFQSTGSLKSTSSSTGAADQVDPGAAAAAAAAAAPPAPPAPAFPPLPPHVATEIDR  
RKKRPLENDGPVKKVKKVQKQEGGSDLGMSGNSEPKCLRTNRVSKSLEKJKEFCDSALPQSRVQTESLQERFAVLPKCTDFD  
DISLLHAKNAVSSSESKRQINQKDTTLFDLSQFGSSNTSHENLQKTASKSANKRSKSIYTPLELQYIEMKQHQHDAVLCVECGYKYR  
FFGEDAEIAARELNICYCHLDHNFMTASIPHTRLFVHVRLVAKGYKGVVVKQTETAAKKAIGDNRSSLFSRKLALYTKSTLIGEDVNP  
LIKLDADVNDDEIMTDTSTSYLLCISENKENVRDKK(QGQHFYWHCGSSAACHRRGCV)

## ACVR2,

## (wt) ORF

MGAAAKLAF VFLJSCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS IEIVKQGCWL  
DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSVLPLMLI AGIVICAFWV  
YRHHKMAYP VLVPTQDPGP PPPSPLLGLK PLQLEVKAR GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG  
MKHENILQFI GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL AYLHEDIPGL KDGHKPAISH  
RDIKSKNVLL KNNLTACIAD FGLALKFEAG KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR  
CTAADGPVDE YMLPFEEIEG QHPSLEDMQE VVVHKKRPV LRDYVQKHAG MAMLCETIEE CWDHDAEARL SAGCVGERIT  
QMQRLTNIIT TEDIVVTVM VTNVDPPPKE SSL\*

A8, Pos. 451: -1 ORF (Mut.rate 16.3%)

MGAAAKLAF VFLJSCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS IEIVKQGCWL  
DDINCYDRTD CVEKKALAKY IFVAVRAICV MKSFLIFRRW KSHSPLQIQL HLSHPITTS SCIPWCHLC\*

A8 Pos. 1476: -1 (Mut.rate 81.6%)

MGAAAKLAF VFLJSCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC YGDKDKRRHC FATWKNISGS IEIVKQGCWL  
DDINCYDRTD CVEKKDSPEV YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSVLPLMLI AGIVICAFWV  
YRHHKMAYP VLVPTQDPGP PPPSPLLGLK PLQLEVKAR GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG  
MKHENILQFI GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL AYLHEDIPGL KDGHKPAISH  
RDIKSKNVLL KNNLTACIAD FGLALKFEAG KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR  
CTAADGPVDE YMLPFEEIEG QHPSLEDMQE VVVHKKRGLF\*

## FLJ11053, A11 Pos. 1695, Mut.rate 52.2%

## wt ORF

MVLRKLSKKD VTTKLKAMQE FGTMCTERDT ETVKGVLPYW PRIFCKISLD HRRRVREATQ QAFELTLKV KKQLAPYLKS  
LMGYWLMACQ DTYTPAAFAA KDAFEAAFP SKOPEAIAFC KDEITSVLQD HLKETPDTL SDPQTVPEEE REAKFYRVVT  
CSLLALKRLL CLLPDNEELS LEEKFKSLLS QNKFWKYGH SVQIRSAFY ELVSALCQRI POLMKKEASK VSPSVLLSID  
DSDPIVCPAL WEAVLYTLT IEDCWLHVNA KKSVPFKLST VIREGGRGLA TVIYPYLLPF ISKLPHSITN PKLDFFKNFL  
TSLVAGLSTE RTTKTSSSESS AVISAFYECL RFIMQQNLGE EEIEQMLVND QLIPFIDAVL KDPGLQHGQL FNHLAETLSS  
WEAKADTEKD EKTAHNLENV LIHFWERLSE ICVAKISEPE ADVESVLGVS NLLQVLQKPK SSLKSSKKGN GKVRFADEIL  
ESNKENEKCV SSEGEKIEGW ELTTEPSLTH NSSGILLSPLR KKPLEDLVCK LADISINYVN ERKSEQHILRF LSTLDSFSS  
SRVFKMLLGD EKQSIVQAKP LEIAKLQKN PAVQFLYQKL IGWLNEDQRK DFGFLVDILY SALRCCDNOM

## -1 ORF

MVLRKLSKKD VTTKLKAMQE FGTMCTERDT ETVKGVLPYW PRIFCKISLD HRRRVREATQ QAFELTLKV KKQLAPYLKS  
LMGYWLMACQ DTYTPAAFAA KDAFEAAFP SKOPEAIAFC KDEITSVLQD HLKETPDTL SDPQTVPEEE REAKFYRVVT

CSLLALKRLL CLLPDNEIDS LEEKFKSLLS QNKFWKYGKH SVPQIRSAFY ELVSALCQRI PQLMKEEASK VSPSVLLSID  
 DSDPIVCPAL WEAVLYTLTT IEDCWLHVNA KKSVPFKLST VIREGGRGLA TVIYPYLLPF ISKLPHSITN PKLDFKKNFL  
 TSLVAGLSTE RTKTSSSESS AVISAFYECL RFIMQQNLGE EEIEQMLVND QLIPFIDAVL KDPGLQHGQL FNHLAETLSS  
 WEAKADTEKD EKTAHNLENV LIHFWERLSE ICVAKISEPE ADVESVLGVS NLLQVLQKPK SSLKSSKKKM VRLDLLMRYL  
 5 KAIKRMKNVYLQKEERLKAG N\*

-2 ORF

MVLRKLSKKD VTTKLKAMQE FGTMCTERDT ETVKGVLPYW PRIFCKISLD HDRRVREATQ QAFEKLTLYV KKQLAPYLKS  
 LMGYWLMACQ DTYTPAAFAA KDAFEAAFP SKQPEAIAFC KDEITSVLQD HLIKETPDTL SDPQTVPEEE REAKFYRVVT  
 0 CSLLALKRLL CLLPDNEIDS LEEKFKSLLS QNKFWKYGKH SVPQIRSAFY ELVSALCQRI PQLMKEEASK VSPSVLLSID  
 DSDPIVCPAL WEAVLYTLTT IEDCWLHVNA KKSVPFKLST VIREGGRGLA TVIYPYLLPF ISKLPHSITN PKLDFKKNFL  
 TSLVAGLSTE RTKTSSSESS AVISAFYECL RFIMQQNLGE EEIEQMLVND QLIPFIDAVL KDPGLQHGQL FNHLAETLSS  
 WEAKADTEKD EKTAHNLENV LIHFWERLSE ICVAKISEPE ADVESVLGVS NLLQVLQKPK SSLKSSKKKW\*

+1 ORF

MVLRKLSKKD VTTKLKAMQE FGTMCTERDT ETVKGVLPYW PRIFCKISLD HDRRVREATQ QAFEKLTLYV KKQLAPYLKS  
 LMGYWLMACQ DTYTPAAFAA KDAFEAAFP SKQPEAIAFC KDEITSVLQD HLIKETPDTL SDPQTVPEEE REAKFYRVVT  
 5 CSLLALKRLL CLLPDNEIDS LEEKFKSLLS QNKFWKYGKH SVPQIRSAFY ELVSALCQRI PQLMKEEASK VSPSVLLSID  
 DSDPIVCPAL WEAVLYTLTT IEDCWLHVNA KKSVPFKLST VIREGGRGLA TVIYPYLLPF ISKLPHSITN PKLDFKKNFL  
 TSLVAGLSTE RTKTSSSESS AVISAFYECL RFIMQQNLGE EEIEQMLVND QLIPFIDAVL KDPGLQHGQL FNHLAETLSS  
 WEAKADTEKD EKTAHNLENV LIHFWERLSE ICVAKISEPE ADVESVLGVS NLLQVLQKPK SSLKSSKKKW\*

10 KIAA1052, A11 Pos. 689, MutRate 42.2%

Wt ORF

MAGRPLRIGD QLVLEEDYDE TYIPSEQUEIL EFAREIGIDP IKEPELMWLA REGIVAPLPG EWKPCQDITG DIYYNFANG  
 QSMWDHPCDE HYRSLVIQER AKLSTSGAIK KKKKKKEKKD KKDRDPPKSS LALGSSLAPV HVPLGGIAPL RGLVDTPPSA  
 25 LRGSQSVSLG SSVESGRQLG ELMPLSQGLK TSAYTKGLG SIYEDKTALS LLGLGEETNE EDEESDNQS VHSSEPLRN  
 LHLDIGALGG DFEYEESLRT SQPEEKDVS LDSAAGPPT PCKPSSPGAD SSLSSAVGKG RQSGGARPGI PEKEENEKSE  
 PKICRNLTVP KADPTGSEPA KASEKEAPED TVDAGEEGSR REEAAKEPKK KASALEEGSS DASQELEISE HMKEPQLSDS  
 IASDPKSFHG LDFGFRSRIE EHLLDVDVLS PVLGGACRQA QQPLGIEDKD DSQSSQDELQ SKQSKGLEER YHRLSPPLPH  
 EERAQSPPRS LATEEPPQG PEGQPEWKEA EELGEDSAAS LSLQLSLQRE QAPSPPAACE KGKEQHSQAE ELGPGQEEAE  
 30 DPEEKVAVSP TTPVSPEVRS TEPVAPPEQL SEAAKAMEE AVAQVLEQDQ RHLESKQEK MQQLREKLQ EEEEEILRIH  
 QKQEQLSSL RERLQKAIEE EEARMREEES QRLSWLRAQV QSSTQADEQ IRAEQEASLQ KLREELESQK KAERASLEQK  
 NRQMLEQLKE EIEASEKSEQ AALNAKEKA LQQLREOLEG ERKEAVATLE KEHSAELERL CSSLEAKHRE VVSSLQKKIQ  
 EAQQKEEAQL QKCLGQVEHR VHOKSYHVAG YEHELSSLLR EKQVEVEGEH ERRLDKMKKE HQQVMAKARE QYEAEEERKQR  
 AELLGHLTGE LERLQRAHER ELETVRQEQH KRLEDLRRRH REQERKLQDL ELDLETRAKD VKARLALLEV QEETARREKQ  
 35 QLLDVQRQVA UKSEEAATATH QQLLEAQKEH THLLQSNQQL REILDELQAR KKLKESQVDL LQAQSQQLQK HFSSLEAEAQ  
 KKQHILLREVT VEENNASPHF EPDLHIEDLR KSLGTNQTKE VSSSLSQSKE DLYLDSLSSH NWWHLLSAEG VALRSAKEFL  
 VQQTRSMRRR QALKAQQH WRHELASAE VAKDPPGIKA LEDMRKNLEK ETRHLDKMS AMRKGHNLLK KKEEKLNLQLE  
 SSLWEEASDE GTLGGSPTKK AVTFDLSDMD SLSSSESSEF SPPLDSTPS LTRKIHGLS HSLRQISSQL SSVLSILDSL  
 NPQSPPLLA SMPAQLPRD PKSTPTPTY GSLARFSALS SATPTSTQWA WDSGQGPRLP SSVAQTVDDF LLEKWRKYFP  
 40 SGIPLLSNP TPLESRLGYM SASEQLRLQ HSHSQVPEAG STTFQGIIEA NRRWLERVKN DPRILPFSST PKPKATLSL  
 QLGLDEHNRV KVVYR\*

-1 ORF

MAGRPLRIGD QLVLEEDYDE TYIPSEQUEIL EFAREIGIDP IKEPELMWLA REGIVAPLPG EWKPCQDITG DIYYNFANG  
 QSMWDHPCDE HYRSLVIQER AKLSTSGAIK KKKKKKRAKT RTETPPKVR WPWVPH\*

-2 ORF

45 MAGRPLRIGD QLVLEEDYDE TYIPSEQUEIL EFAREIGIDP IKEPELMWLA REGIVAPLPG EWKPCQDITG DIYYNFANG  
 QSMWDHPCDE HYRSLVIQER AKLSTSGAIK KKKKKGKERQ EGQRPPQKFA GLGFLISPSS CSSWGPFSFT RSCGYPTLCS  
SWISKREPGELSGVWTSARW THAAFTGSQD LCLYKGSGLH\*

+1 ORF

50 MAGRPLRIGD QLVLEEDYDE TYIPSEQUEIL EFAREIGIDP IKEPELMWLA REGIVAPLPG EWKPCQDITG DIYYNFANG  
 QSMWDHPCDE HYRSLVIQER AKLSTSGAIK KKKKKGKERQ QEGQRPPQKF AGLGFLISPSS SCSSWGPFSF TRSCGYPTLC  
SSWISKREPGELSGVWTSARW THAAFTGSQD LCLYKGSGLH\*

Figure 3

## FSP02

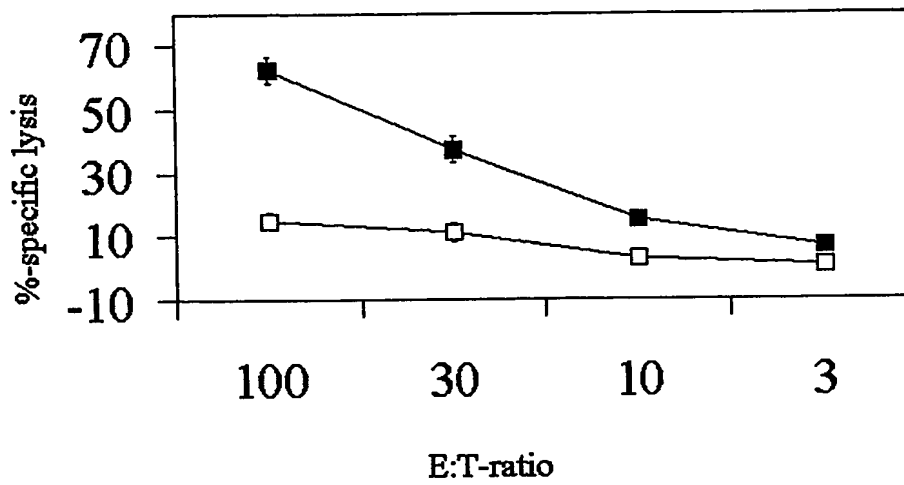
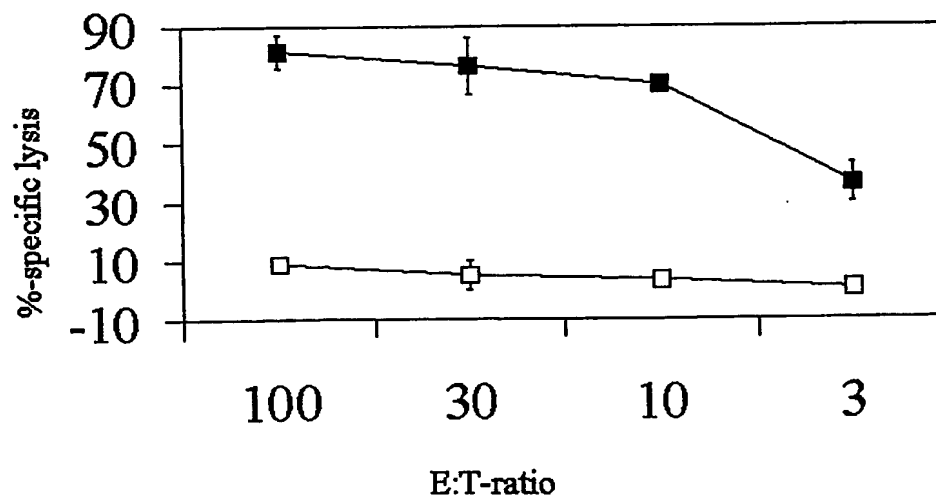


Figure 4

## FSP06



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/04083

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C07K14/82 G01N33/48 A61K48/00 A61K38/00 C12Q1/68		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07K G01N A61K C12Q		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the International search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, SEQUENCE SEARCH, CHEM ABS Data		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EST, 10 September 2001 (2001-09-10) NATIONAL INSTITUTES OF HEALTH: "60233245139F1 NIH_MGC_96 Homo sapiens cDNA clone IMAGE:5287496 5', mRNA sequence" Database accession no. BI603282 XP002226919 abstract	1
X	DATABASE EMBL 'Online! EST, 15 March 2002 (2002-03-15) NATIONAL INSTITUTES OF HEALTH: "AGENCOURT _6621833 NIH_MGC_125 Homo sapiens cDNA clone IMAGE:5724573 5', mRNA sequence" Database accession no. BM906641 XP002226916 abstract	1
-/-		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the International filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the International filing date but later than the priority date claimed "T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the International search  4 September 2003		Date of mailing of the International search report  08.12.03
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  Guarinos Viñals, E

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/04083

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL 'Online! EST, 8 March 2002 (2002-03-08) KIM N.S.: "K-EST0027113 S9SNU601 Homo sapiens cDNA clone S9SNU601-25-D02-5', mRNA sequence" Database accession no. BM751114 XP002226917 abstract</p>	1
X	<p>DATABASE EMBL 'Online! EST, 11 February 2001 (2001-02-11) GENOSCOPE: "5 prime end of clone CSODI023YL23 of library LTI_NFL006_PL2 from placenta of male Homo sapiens (human)" Database accession no. AL545813 XP002226918 abstract</p>	1
X	<p>DATABASE EMBL 'Online! EST, 30 September 2000 (2000-09-30) NATIONAL INSTITUTES OF HEALTH: "601510256F1 NIH_MGC_71 Homo sapiens cDNA clone IMAGE:3912036 5', mRNA sequence" Database accession no. BE883852 XP002226920 abstract</p>	1
X	<p>WO 02 04664 A (KNEBEL DOEBERITZ VON MAGNUS ;LINNEBACHER MICHAEL (DE); BORK PEER ()) 17 January 2002 (2002-01-17) examples 1-8 page 6, last paragraph page 7, paragraph 2</p>	1-19, 22-36, 39-42
X	<p>LINNEBACHER M ET AL: "FRAMESHIFT PEPTIDE-DERIVED T-CELL EPITOPES: A SOURCE OF NOVEL TUMOR-SPECIFIC ANTIGENS" INTERNATIONAL JOURNAL OF CANCER, NEW YORK, NY, US, vol. 93, 6 April 2001 (2001-04-06), pages 6-11, XP001061478 ISSN: 0020-7136 the whole document</p>	1-11,18, 19,22-31
A	<p>WOERNER S M ET AL: "SYSTEMATIC IDENTIFICATION OF GENES WITH CODING MICROSATELLITES MUTATED IN DNA MISMATCH REPAIR-DEFICIENT CANCER CELLS" INTERNATIONAL JOURNAL OF CANCER, NEW YORK, NY, US, vol. 93, no. 1, 1 July 2001 (2001-07-01), pages 12-19, XP001083712 ISSN: 0020-7136</p>	

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# INTERNATIONAL SEARCH REPORT

Int. Patent Application No.  
PCT/EP 03/04083

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DI PIETRO C ET AL: "Genomic localization of the human genes TAF1A, TAF1B and TAF1C, encoding TAFI48, TAFI63 and TAFI110 subunits of class I general transcription initiation factor SL1." CYTOGENETICS AND CELL GENETICS, vol. 89, no. 1-2, 2000, pages 133-136, XP009003813 ISSN: 0301-0171</p> <p>-----</p>	

## INTERNATIONAL SEARCH REPORT

ational application No.  
PCT/EP 03/04083

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 5-10, 18-29 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  

1-19, 22-36, 39-42 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-19, 22-36, 39-42 partially

a polynucleotide with the insertion or deletion of one or two A in the A11 repeat of the gene TAF1B, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them

2. Claims: 1-19, 22-36, 39-42 partially

a polynucleotide with the insertion or deletion of one or two A in the A11 repeat of the gene MACS, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them

3. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two A in the A10 repeat of the gene UVRAG, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

4. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two G in the G9 repeat of the gene ELAVL3, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

5. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two A in the A10 repeat of the gene TCF6L1, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

6. Claims: 1-19, 22, 25-36, 39-42 partially

a polynucleotide with the insertion or deletion of one or two A in the A10 repeat of the gene ABCF1, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. Claims: 1-19, 22, 24-36, 39-42 partially

a polynucleotide with the insertion or deletion of one or two A in the A10 repeat of the gene AIM2, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

8. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two A in the A10 repeat of the gene CHD2, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

9. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two A in the A11 repeat of the gene FLJ11053, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

10. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two A in the A11 repeat of the gene KIAA1052, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

11. Claims: 1-17 partially

a polynucleotide with the insertion or deletion of one or two A in the A8 repeat of the gene ACVR2, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

12. Claims: 1-19, 23-31 partially

a polynucleotide with the insertion or deletion of one or two A in the A11 repeat of the gene HT001, a polypeptide encoded by said polynucleotide, a method of treatment or detection involving them and a pharmaceutical composition or a diagnostic kit comprising them.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

13. Claims: 3-19, 22-36, 39-42 partially

a frameshift polypeptide U79260, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

14. Claims: 3-19, 22, 23, 25-36, 39-42 partially

a frameshift polypeptide PTHL3, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

15. Claims: 3-19, 23-36, 40-42 partially and 20, 37 completely

a frameshift polypeptide TGFbRII, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

16. Claims: 3-19, 22, 23, 25-36, 39-42 partially

a frameshift polypeptide TCF4, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

17. Claims: 3-19, 22-36, 39-42 partially

a frameshift polypeptide AC1, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

18. Claims: 3-19, 22, 23, 25-36, 39-42 partially

a frameshift polypeptide Sec63, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

19. Claims: 3-19, 22-36, 39-42 partially

a frameshift polypeptide CASP5, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

20. Claims: 3-17 partially

a frameshift polypeptide USLC23A1, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

21. Claims: 3-17 partially

a frameshift polypeptide HSPC259, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

22. Claims: 3-19, 25-36, 40-42 partially and 21, 38 completely

a frameshift polypeptide BAX, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

23. Claims: 3-17 partially

a frameshift polypeptide FTL3L, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

24. Claims: 3-19, 24-31 partially

a frameshift polypeptide OGT, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

25. Claims: 3-17 partially

a frameshift polypeptide MAC30X, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

26. Claims: 3-17 partially

a frameshift polypeptide SLC4A3, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

27. Claims: 3-17 partially

a frameshift polypeptide PRKDC, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

28. Claims: 3-19, 24-31 partially

a frameshift polypeptide MSH3, a method of treatment or detection involving it and a pharmaceutical composition or a

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

diagnostic kit comprising it.

29. Claims: 18, 19, 22-36, 39-42 partially

a frameshift polypeptide IGFIIR, a method of treatment or detection involving it and a pharmaceutical composition or a diagnostic kit comprising it.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 03/04083

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